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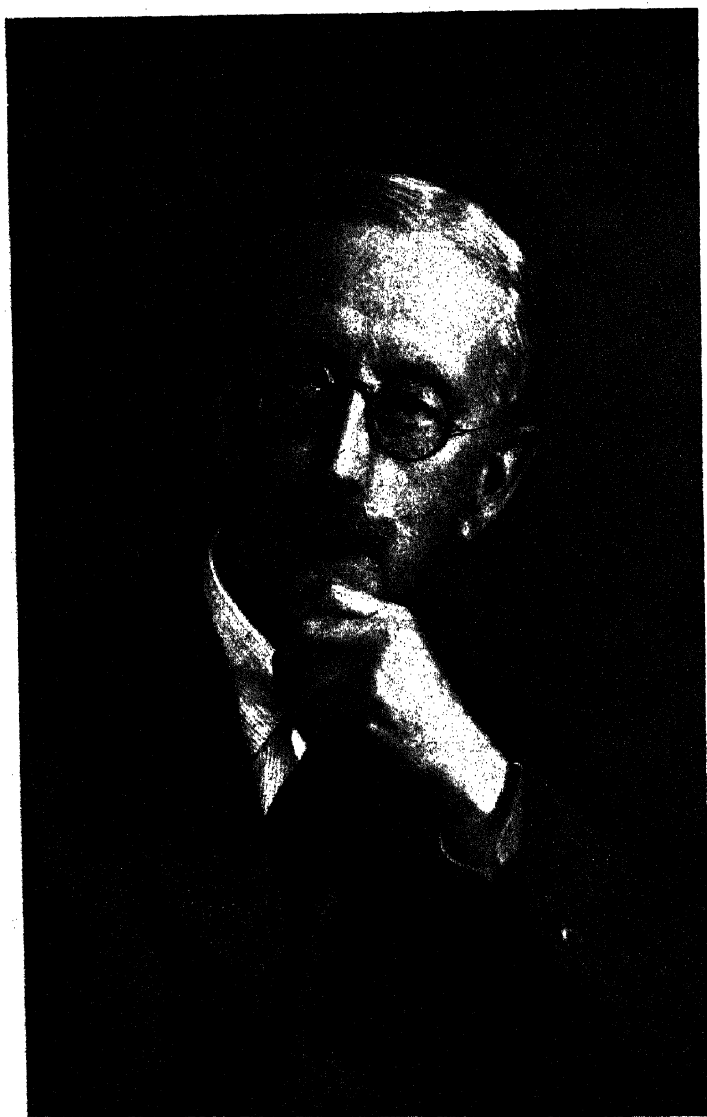
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*Edwin S. Goodrich*

*Emery Walker Ed. phic*

*Professor E. S. Goodrich, F.R.S.*

THE death of Professor E. S. Goodrich has taken from us a great comparative anatomist who for a quarter of a century was the Editor of this Journal. A whole generation of contributors will remember his kindly help and advice in the publication of their work. He became Editor of the 'Q.J.' in succession to Sir E. Ray Lankester in 1920, and his editorship fittingly ended with the publication in the latest volume of a grand survey of that field of biological interest with which his name will always be particularly associated. An obituary notice by Professor A. C. Hardy will be published in this Journal.

The newly appointed Editors will do all in their power to maintain the high standards of their predecessors. They look particularly for the support of those who are now returning to active biological work on the conclusion of the war.

The Journal has a great tradition for the publication of accurate researches in micro-anatomy leading to fundamental advances in knowledge. In actively upholding this tradition, it will be the policy of the Editors to give particular encouragement to those micro-anatomical studies that throw light on physiological and functional problems of all kinds.

There is another class of work in which the Editors are anxious to see a very considerable expansion. The Journal has been since its early days a medium for the publication of what may broadly be called cellular biology. Lankester himself was a pioneer in some parts of this field. A period now lies before us when great advances are to be expected in our interpretation of cellular organization and in our knowledge of the

fine structure of biological systems, especially in the fields of strict histochemistry and cytochemistry—the biochemistry and biophysics of cell-structure *in situ*. The Editors wish to give every encouragement to contributions in these fields of inquiry.

The advance in cellular studies is leading to new methods of investigation and to new developments in microscopical apparatus. The Editors will be glad to consider the publication of papers on these subjects.

Contributors to the Journal are asked to address papers offered for publication to the Editors, Q.J.M.S., and to direct them to Dr. C. F. A. Pantin, Zoological Laboratory, The University, Cambridge.

C. F. A. PANTIN

JOHN R. BAKER

# The Development of the Pituitary of the Laboratory Mouse.

By

T. Kerr,

Department of Zoology, University of Leeds.

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With 6 Text-figures and Plates 1, 2, and 3.

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## INTRODUCTION.

THE development of the pituitary has been studied thoroughly in a few mammals (e.g. Frazer, 1916, in marsupials; Atwell, 1918, in the rabbit; Brahms, 1932, in the cat; Kingsbury and Roemer, 1940, in the dog) and many are known in less detail, so that the general outlines of its embryology are understood. A number of aspects, however, still require elucidation. The mouse was chosen as it has not previously been described and as the beginning of pregnancy is sufficiently predictable in this form for a plentiful supply of embryos to be obtained at any required stage.

Since Minot (1897) pointed out the close and persistent contact between mouth and brain established in the hypophysial region during early development the importance of the condition has received sufficient emphasis (Kingsbury and Adelmann, 1924; Brahms, 1932; Gilbert, 1934 and 1935; Kingsbury and Roemer, 1940) and it is unnecessary to discuss the alternative view that the gland forms as two separate diverticula. However, information concerning these early stages is scanty and accordingly a mechanical explanation, namely that Rathke's pouch is a passive nipping off of part of the mouth epithelium which results from surrounding growth changes (Mihalkovics, 1877; Adelmann, 1922; Kingsbury and Adelmann, 1924; Hochstetter, 1924), may in turn be carried too far. Hence information is especially needed of stages previous to the rupture of the oral membrane, of changes in cranial flexure and those associated with separation of brain and endodermal gut, of the time of delimitation and the behaviour of the hypophysial plate, of the



nature and ultimate fate of the brain to mouth contact, and of the details of closure of Rathke's pouch.

The origin and growth of the nervous lobe is not nearly so well understood as that of the glandular component. Most authors accept its separate formation as a diverticulum of the infundibular region of the brain and merely describe its condition at different stages, though Mihalkovics (1875) suggested that the vesicle was the result of upward pressure of the pouch on the brain floor and Hochstetter (1924) that it was due to very active growth of the postoptic lamina. Gilbert (1934), in a detailed study of the lobe in the cat, finds a virtual disappearance of mitoses from the early infundibular region and explains the formation of the vesicle by the interaction of surrounding tissues—in particular the rotation of the optic and infundibular regions about the mouth to brain contact—again a mainly mechanical solution with the lobe tissue essentially passive. Later growth she suggests is due to the addition of cells from the adjoining brain walls, and Kingsbury and Roemer (1940) have seen evidence of such a migration in the dog. This region in the mouse has been examined by the use of sections in all planes and when possible by arresting mitotic activity with colchicine.

In addition there remain various details concerning the lateral lobes, the blood-supply, and cell-differentiation, the incidence of mitosis and general architecture, on which more information is desirable. The interdependence of the various parts of the pituitary gland has made a description of complete stages preferable to one of each part separately.

#### MATERIALS AND METHODS.

The mice used for this work were kept in pairs at about 70° F. on a mixed diet. To obtain the embryos needed at any particular stage the young of the first pregnancy were removed the morning of their birth and the male the following morning, so that the latter date could be taken as the first day of the new pregnancy. At the required time the mother was killed and the embryos fixed in Susa mixture, later embedded by Peterfi's method, sectioned at 6 $\mu$  and stained in Mayer's haemalum. The head to rump measurements of the embryos are given, after the somites

become obscured, and their corresponding external characters are in accordance with Gruneberg's (1948) list.

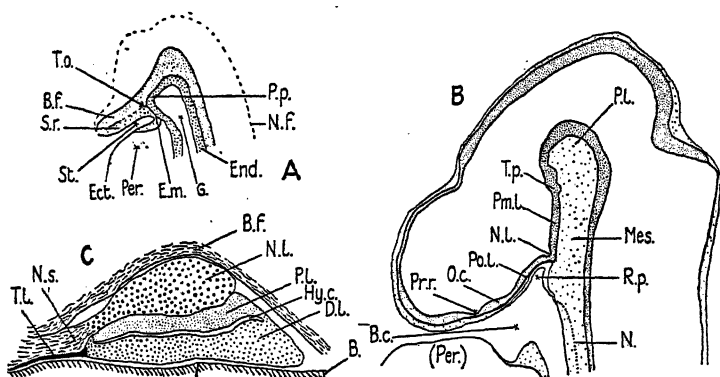
Colchicine treatment of embryos at all stages was intended, to allow mitotic counts and indicate growth areas, but did not prove possible. The injection of 0.1 mg. of colchicine in aqueous solution into the mother  $9\frac{1}{2}$  hours before killing gives living embryos showing the colchicine effect from 16 days old (about 15 mm.) till birth on the morning of the nineteenth day, but younger embryos show necrosis. Half of this dosage gives embryos which are alive and show the colchicine effect down to 15 days old (about 12 mm.), but for earlier embryos this too is generally fatal and it did not prove possible to get consistent effects with smaller dosages. The necrotic embryos are valueless for counts but show the distribution of mitoses.

#### GENERAL CONSIDERATIONS.

The development of the pituitary should be considered in relation to that of the anterior region of the body as a whole, and an outline of the relationships of this region and of the nomenclature adopted may avoid confusion. Gilbert (1935, in the rat and man) has described the earlier stages of development from the flattened embryonal disc stage up to the closure of the neuropore and the establishment of the fully formed ectodermal stomodaeum, separated from the endodermal foregut by the oral membrane (the enterostomial membrane of Goodrich, 1935). Briefly, the anterior border of the neural plate grows more rapidly at the sides than in the centre, resulting in the appearance of two lateral folds with a 'terminal notch' between. The lateral folds become connected across the mid-line by a solid band of ectoderm—the torus opticus—in which neither neural nor ordinary surface ectoderm can at first be distinguished, but as the band grows forwards these two layers separate out posteriorly. Since the torus opticus appeared initially just anterior to the endodermal foregut the neural tissue derived from it forms the floor of the brain as far as the neuropore whilst the corresponding surface ectoderm forms the roof of the stomodaeum from the enterostomial membrane forwards (Text-fig. 1 A). The differentiation of the two layers is neither immediate nor complete and

in the posterior part, just in front of the enterostomial membrane, there probably persists the adherence between brain and ectoderm which is so important in the later development of the pituitary. This is the stage at which the description of the development of the pituitary in the present work begins.

When the enterostomial membrane breaks down and the ectodermal stomodaeum becomes indistinguishably continuous with the beginning of the endodermal foregut to form the buccal



TEXT-FIG. 1.

Diagrams to show relationships and the nomenclature adopted.

A, Sagittal section of the head-end of a 5-somite embryo. B, Sagittal section of the head-end of a 4½-mm. embryo. C, Sagittal section of the adult pituitary gland. Head-end to the left as in all text-figures. B, bony base of skull; Bc, buccal cavity; Bf, brain floor; Dl, distal lobe; Em, enterostomial membrane (oral membrane); Ect, ectoderm; End, endoderm; G, foregut; Hyc, hypophysial cavity; Mes, mesoderm; N, notochord; Nf, edge of neural folds (out of section); Nl, nervous lobe; Ns, neural stalk; Oc, optic chiasma; Pl, proximal lobe; Pp, prechordal plate; Per, pericardium; Pl, plica; Pml, premammillary lamina; Pol, postoptic lamina; Prr, preoptic recess; Rp, Rathke's pouch; Sr, roof of stomodaeum; St, stomodaeum; Tl, tubal lobe; To, position of the torus opticus in earlier development; Tp, tuberculum posterius.

cavity, the structures in the hypophysial region are becoming defined (Text-fig. 1 B). The fold, or plica (Pl) separating off the hind-brain shows near the top of its anterior face the tuberculum posterius (Tp) downwards from which stretches the premammillary lamina (Pml) connecting the optic thalami to

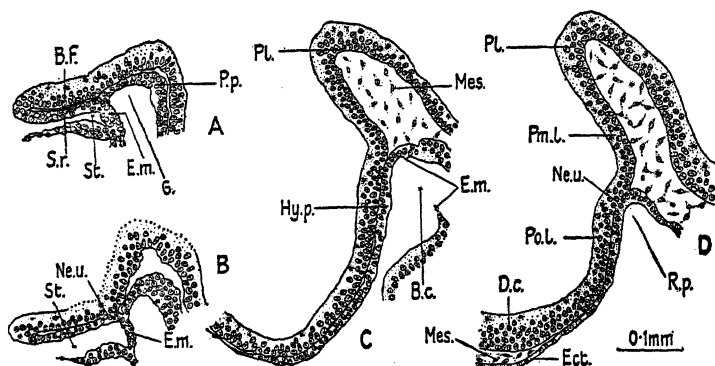
either side and terminating ventrally in the infundibular region from which develops the nervous lobe (*Nl*). The postoptic lamina (*Pol*) extends forwards from the nervous lobe as far as the thickening of the optic chiasma (*Oc*), bounded in front by the preoptic recess (*Prr*). To the nervous lobe is attached Rathke's pouch (*Rp*), the ectodermal pocket from the roof of the buccal cavity (*Bc*) from which part of the pituitary develops. The pouch is still open to the buccal cavity, later it closes but remains for a time attached to the buccal roof by the hypophysial (or buccal) stalk.

Various descriptive terms have been used for the adult gland, based on different interpretations and the use of different animals. The term 'pituitary' was due originally to the theory that the gland produced the nasal secretion, whilst the term 'hypophysis' is one in a system of nomenclature applied to a series of structures associated with the brain. Both names referred to the complete adult organ, and are still widely used synonymously, so that there appears to be no advantage in giving either of them a second and arbitrary meaning. The gland itself (Text-fig. 1 c) is formed of a nervous component consisting of the nervous lobe (*Nl*) derived from the brain of the embryo and still connected to it by the neural stalk (*Ns*), and of a glandular component which is unquestionably secretory and derived from the tissue of Rathke's pouch. The glandular component again has in most forms a layer of histologically distinct tissue attached to the nervous lobe and here accordingly called the proximal lobe (*Pl*) (also known as the intermediate lobe), a hypophysial cavity (*Hyc*) representing the cavity of Rathke's pouch, a larger mass of characteristic tissue called here the distal lobe (*Dl*) in contrast to the proximal (this is also known as the anterior lobe but is variable in position, in the mouse it is ventral), and the tuberal lobe (*Tl*) formed from the fused tips of the lateral lobes of the embryo.

#### DESCRIPTION.

Stage 1, up to about 9 days old, sixteen somites, covering the appearance of the hypophysial plate and the first stage in the development of Rathke's pouch.

An embryo of six somites, or almost eight days old, still shows the 'inversion of the germ layers' and a widely open brain (see Snell, 1941, for details of general embryology). The paraxial mesoderm has penetrated laterally into the head region, but there is a mid-line area free of mesoderm between the brain floor and the roof of the stomodaeum (fig. 1, Pl. 1; Text-fig. 2 A). These



TEXT-FIG. 2.

Sagittal sections of hypophyseal area and related structures. A, 6-somite embryo; B, 12-somite embryo; C, 16-somite embryo; D, 2½-mm. embryo (about 21 somites). *Hyp*, hypophyseal plate; *Neu*, neuro-ectodermal union. Other lettering as before.

two layers lie close against each other in the mid-line, doubtless the result of their method of development (Gilbert, 1935), but are quite distinguishable anteriorly; posteriorly they are very closely apposed and in two out of the five embryos examined there is a small area of apparent fusion a little in front of the enterostomial membrane. The ectoderm of the stomodaeal roof is homogeneous in thickness and flat so that no separate hypophyseal plate can be defined. Behind the enterostomial membrane the top of the foregut is formed by the thickened prechordal plate which is still in contact with the brain; passing back from the prechordal plate is the notochord, still imperfectly differentiated from the gut roof. Here, too, no mesoderm has penetrated into the mid-line.

The turning of the embryo to correct the 'inversion of the

germ layers' is accomplished in the anterior region by the ten- to twelve-somite embryo, about  $8\frac{1}{2}$  days old, but the brain is still open in front and the roof of the stomodaeum flat and homogeneous in sagittal section. Brain floor and stomodaeal roof remain distinct anteriorly but behind they are still very closely apposed. In three series there is a small area of apparent fusion in front of the enterostomial membrane (fig. 2, Pl. 1; Text-fig. 2 B), in two further series actual fusion is doubtful. This phenomenon was first pointed out by Minot (1897) and later emphasised among others by Gilbert (1934); the term 'neuro-ectodermal union', used by the latter, is restricted here to the posterior area where apparent fusion or very close contiguity occurs. A close relation between brain and stomodaeum is present in this region from the first (Gilbert, 1935), but it is possible that the actively growing optic regions above and in front and preammillary region above and behind emphasize it posteriorly by increased pressure. The enterostomial membrane is still intact, though its ectodermal layer is thin; the prechordal plate is distinct and breaking away from the brain, but no mid-line mesoderm has yet appeared.

The actively dividing mesoderm which is moving into the head mainly from the trunk region penetrates laterally without hindrance, but in the mid-line its passage is impeded by the meeting of brain and stomodaeum and possibly by the prechordal plate from which the mesoderm-free area stretches forwards to the present anterior end of the embryo. Later this area too is invaded on either side from in front backwards to about the anterior border of the hypophysial area, but it is much later before any mesoderm penetrates as far back as the neuro-ectodermal union (Text-figs. 2 and 3). This increase in mesoderm separates the brain from the surface ectoderm and possibly, along with the rapid growth of the brain, induces a stretching influence upon the latter. Certainly the lower head ectoderm attenuates greatly during the first two stages described here except over certain defined areas, one of which is the hypophysial; this area retains its thickness partly because the mitotic rate is higher and partly perhaps because it may now be determined as hypophysial tissue. In any case attenuation spreads

backwards until by the end of this stage the anterior edge of the hypophysial plate is becoming faintly defined. Later the ectoderm of the plate may thin slightly but on the whole it retains the primitive condition of the dorsal stomodaeal epithelium. Laterally the margins of the plate are obscured until later by other localized thickenings; posteriorly the plate ends at the enterostomial membrane.

In the fourteen-somite embryo the enterostomial membrane is beginning to break down and as a result the stomodaeum is becoming continuous with the upper end of the foregut to form the buccal cavity. Meantime the growth of the nervous tissue has given rise to the plica, the notochord is differentiating from the gut and the prechordal plate as such is disintegrating, so that a gap appears between the foregut and the hind-brain and spinal cord which is rapidly invaded by mesoderm. At the same time the part of the hypophysial plate which lies behind the neuro-ectodermal union, and which meanwhile has grown in extent, is bent backwards to retain contact with the endoderm, so producing in sagittal section the posterior face of an ectodermal pocket attached at its tip to the brain and open below to the newly formed buccal cavity (Text-fig. 2 c and d). This shallow pocket is the first stage in the development of Rathke's pouch. The most important factor involved in its establishment appears to be the rapid growth of the nervous tissue, with the bending of the hypophysial plate and the appearance of the mid-line mesoderm following as a result. The longer and thinner anterior face of the pocket is bent down by the growth of the forebrain only, the anterior mesoderm being too scanty to have any effect. The hypophysial cells are now more or less columnar, with the nuclei towards their inner ends, so that there is a zone of cytoplasm on the free surface; it is in this zone almost exclusively that mitoses occur, giving the impression that the nuclei move out into it, divide and move back again.

Stage 2, nine to ten days old, about 2 to 4½ mm. tail-rump length, covering the conversion of the open pocket into a deep pouch and the appearance of the infundibular recess.

This stage is characterized by the great and rapid increase of mesoderm around the hypophysial region, particularly laterally

and posteriorly. Considered only in sagittal section (e.g. fig. 5, Pl. 1) the pouch might appear to be forming entirely under the influence of external factors, but in horizontal section (i.e. roughly longitudinal to the long axis of the pouch) its sides are closing in surrounded only by loose embryonal mesoderm. The maxillary processes, suggested by Haller and Mori (1925) as conditioning lateral closure of the pouch, are too far out for their mesodermal concentrations to have any direct effect.

The hypophysial plate was at first flat, then dome-shaped, now the edges approach each other and it becomes tubular; this reorganization occurs without the appearance of folds, the pouch remaining approximately circular in cross section (fig. 4, Pl. 1) up to about 3 mm. embryo length, so that a constant rearrangement of the hypophysial cells must be going on. This must free a proportion of the cells for use in the elongation of the pouch while at the same time there is constant mitosis in the hypophysial tissue, so that there appears to be sufficient tissue available from these sources to enable pouch formation to proceed. In other words, instead of considering this part of development as the automatic nipping off of part of the stomodaeal epithelium by external forces, it might be better described as the passive formation of a pouch by an active adjustment of already determined hypophysial tissue which keeps pace with and is aided by neighbouring growth changes. The hypophysial tissue becomes increasingly well defined as the ectoderm and endoderm attenuate, although a transitional border of cells remains. There is never any attachment of notochord to pouch wall in the mouse, nor any suggestion that the endoderm may play any part in wall formation.

The neuro-ectodermal union, apart from any inductive significance it may have, can clearly function as a point of attachment for the formation of Rathke's pouch. If the formation of the pouch is mechanical the main factors generally considered responsible (see Gilbert, 1934)—cranial flexure and increase in mesoderm—will act upon it in different ways. For cranial flexure only a weak attachment will be necessary. For increase in mesoderm however a distinct pull at this point might be expected, as the adjacent ectoderm and endoderm are forced away from the brain, but some observations suggest the reverse. On

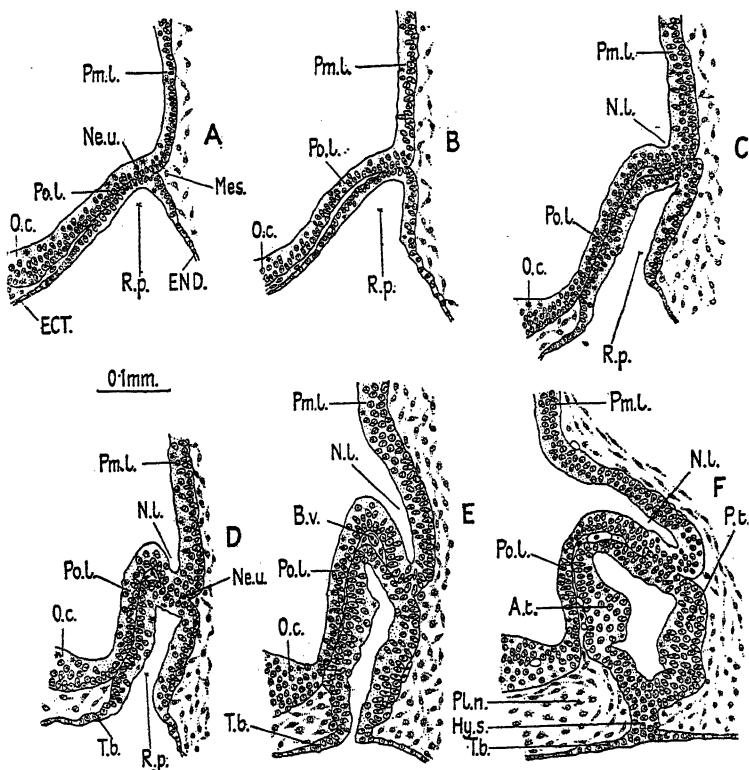


either side of the mid-line just anterior to the union the pouch tissue is pushing up beside the brain (fig. 3, Pl. 1), also the pouch is thinner in the mid-line where it appears to be pressing on the brain, and finally the nervous lobe appears first just above the union.

The union itself remains as before, always close and sometimes apparently a true fusion. In ten-day embryos which have been killed by colchicine in utero the hypophysial and brain tissues have parted leaving a 'membrane' between, so that an intervening basement membrane may normally exist, as in the armadillo (Oldham, 1941). The union is restricted in area, extending over a few sections in either plane, and its relative position begins to change in about the  $3\frac{1}{2}$  mm. embryo from the top front of the pouch to a more posterior position. The union persists during this alteration and the inner end of the pouch appears to be turned over; one effect is to equalize the lengths of the anterior and posterior faces of the pouch so that when the edges later meet they do so squarely (Text-fig. 3).

Up to about 3 mm. embryo length there is little difference in thickness between the premammillary lamina and the brain wall to either side (Text-fig. 4 A), and mitoses are common throughout. The lamina at  $3\frac{1}{2}$  mm. as seen in transverse section is becoming marked off as a curved region of roughly homogeneous thickness with fewer mitoses, and as growth proceeds it becomes increasingly distinct as a relatively thin area between the mitotically very active and rapidly thickening sides of the brain. Between  $3\frac{1}{2}$  and 4 mm. embryo length the lower end of this lamina becomes grooved (fig. 1, Pl. 2; Text-fig. 4 B), just above the neuro-ectodermal union. The groove has deepened and enlarged by the end of this stage into a gutter, oriented upwards from the union and fading away above into the ordinary premammillary lamina. This is the first appearance of the infundibular recess and so of the nervous lobe of the pituitary. Mitotic activity in the postoptic lamina is still common and from sagittal sections only it might be supposed that active growth of this lamina was indeed the factor responsible for the recess (Hochstetter, 1924). Possibly it may play a minor part in the initiation of the groove. The rotation of the pouch tip mentioned

previously appears more likely to be due at first to the formation of the gutter and later to differential growth of the upper front pouch wall.



TEXT-FIG. 3.

Sagittal sections to show closure of Rathke's pouch. A, 3-mm. embryo; B, 3½-mm.; C, 4½-mm.; D, 5-mm.; E, 5½-mm.; F, 7½-mm. *At*, anterior wall thickening; *Bv*, blood-vessel; *Hys*, hypophysial stalk; *Pt*, posterior wall thickening; *Pln*, prechondral nuclei; *Tb*, transitional border of cells. Other lettering as before.

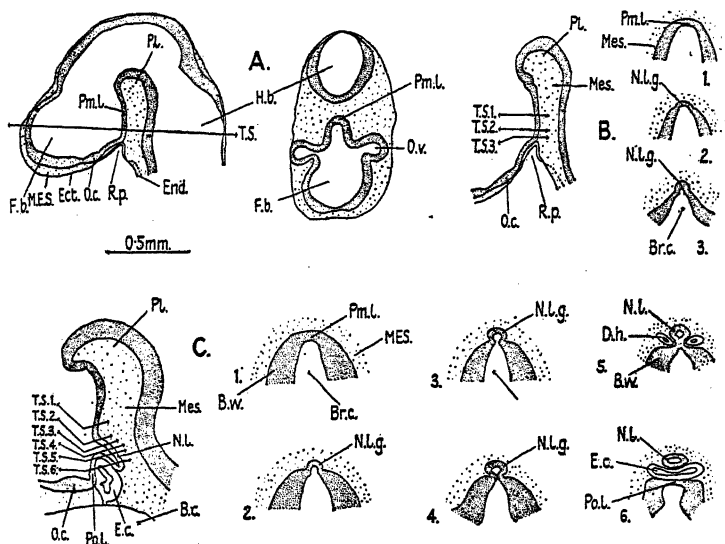
Stage 3, eleven days old, 4½ to 7 mm. in length, covering the closure of the pouch and the formation of the nervous lobe.

In the later phases of pouch formation its anterior wall continues to be pushed back to some extent by the brain floor, but

elsewhere only loose mesoderm surrounds the pouch (Text-fig. 3). The prechondral nuclei below the brain become distinguishable as they round themselves off in about the 7-mm. embryo (fig. 6, Pl. 2; Text-fig. 3 r) with capsules of cartilage not identifiable till about 8 mm. Hence the actual closure of the pouch at about 6 mm. can hardly be under the influence of either mesodermal concentration or cartilage formation and must be considered to occur under the control of the pouch tissue itself. In the mouse only the bottom edges of the pouch walls meet, close to the buccal roof, so that the stalk is at first short and formed largely of the transitional border of cells. Part of this border however persists as a thickened area of buccal ectoderm which usually thins later and becomes gradually indistinguishable. During this stage the long axis of the pouch is still roughly in line with the pream-millary lamina and at right angles to the adjoining buccal epithelium. In transverse section the pouch begins to change in shape from circular to oval at about  $3\frac{1}{2}$  mm. embryo length and by 7 mm. this is becoming marked. Also from about 5 mm. two horns grow up rapidly from the top anterior edge of the pouch, one on either side of the developing nervous lobe and close to the brain. They contain prolongations of the hypophysial cavity and mitotically this region is the most active part of the pouch.

The nervous lobe takes recognizable shape during this stage and it is now that the problems concerning its establishment arise. The appearances in the mouse might support all the previously mentioned views regarding its formation in some degree, but the main process involved is quite different. The nervous lobe is formed here by the gutter in the lower pream-millary lamina, which appeared during the last stage, deepening (fig. 2, Pl. 2) and its edges meeting and fusing (figs. 3 and 4, Pl. 2; Text-fig. 4 c). The fusion starts just above the neuro-ectodermal union, where the nervous tissue remains attached to the pouch, and as it proceeds upwards the walls separate as the anterior face of the nervous lobe and the upper part of the postoptic lamina. The process is easily followed in transverse sections, i.e. sections cut at right angles to the pream-millary lamina; in longitudinal sections it is not apparent. In its earlier condition the gutter gradually died away when traced up the pream-

millary lamina, but by the 7-mm. embryo its upper end has become quite precise and the presumptive nervous lobe tissue thereby delimited. Fusion of the gutter walls proceeds until there remains only a circular opening by which the nervous lobe



TEXT-FIG. 4.

Diagrams to illustrate the development of the nervous lobe. A sagittal section is shown for reference in each case, with the positions of the corresponding transverse sections indicated. A, Sagittal and transverse section of the entire head region of a 3-mm. embryo. B, Sagittal section of the plica and the hypophyseal region only of a 4-mm. embryo, with transverse sections to show the first appearance of the nervous lobe gutter at the lower (infundibular) end of the premammillary lamina. C, Sagittal section of the plica and the hypophyseal region of a 7½-mm. embryo, with transverse sections to show different degrees of closure of the gutter at different levels. *Bw*, brain wall; *Brc*, brain cavity; *Dh*, dorsal horn; *Ec*, ectodermal component of pituitary; *Fb*, fore-brain; *Hb*, hind-brain; *Nlg*, nervous lobe gutter; *Ov*, optic vesicle. Other lettering as before.

cavity communicates with the cavity of the brain. As a result of its method of origin the lobe is at first roughly parallel to the premammillary lamina above, but very soon its orientation

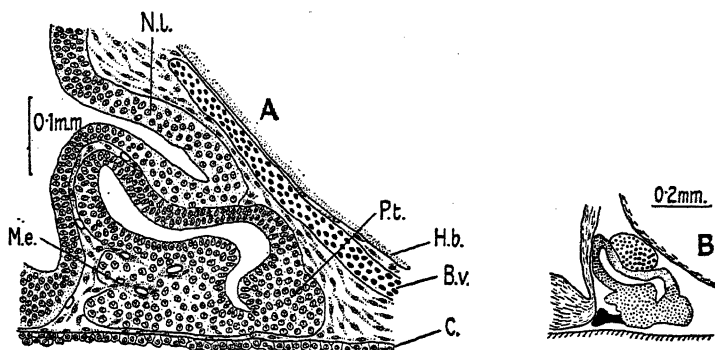
commences to alter under pressure from the pouch tissue below.

Stage 4, twelve to fourteen days old, 7 to 11 mm. in length, covering the reorientation of the gland and the thickening of its walls, the formation of the lateral lobes and the cell cords, and the establishment of the blood supply.

The hypophysial stalk reaches its maximum length between 7- and 8-mm. embryo length and ruptures between 8 and 9 mm., remnants of the stalk persisting in a few individuals as in other mammals. At about  $7\frac{1}{2}$  mm. localized thickenings (fig. 6, Pl. 2) appear on the anterior and posterior faces of the pouch, the former a little above the latter, so that the originally straight hypophysial cavity becomes bent into an elongated S-shape. The thickenings are not due to a localized increase in mitotic activity, which indeed is lower in these regions, but to a readjustment within the tissue itself. The earlier condition of the walls, persisting still in their upper parts and in the horns, is for the nuclei to be somewhat elongated and oriented roughly at right angles to the hypophysial cavity, for the cytoplasm to be quite dense, and for mitoses normally to be confined to the nuclei adjacent to the cavity. In the thickenings the nuclei have moved outwards and apart, rounded themselves off, and the cytoplasm has become less dense. The process commences in the cells farthest from the cavity, and for some time the remains of the original wall can be distinguished; some time too elapses before mitosis is at all common in this looser tissue away from the edges of the cavity. Later this loosening effect spreads round the base of the pouch tissue and then up either side.

By 8-mm. embryo length a reorientation of the gland has begun. The underlying cartilage, in which no sella turcica develops, is becoming sufficiently formed to prevent any enlargement ventrally, dorsally the nervous lobe can be forced up somewhat, and to the front and rear a small amount of space is available. Meanwhile the anterior thickening is pushing out the large, blunt and irregularly shaped processes which represent the anterior 'cell cords' in this form. As a result of this differential growth the main axis of the hypophysial cavity is being turned through  $90^\circ$  backwards and upwards (Text-figs. 3 F, 5 A

and 6), carrying the upper pouch wall and the nervous lobe along with it, so that the relative dispositions of the adult gland (Text-fig. 1 c) gradually become apparent. The main increase in bulk, however, must be by lateral expansion, into regions occupied only by loose mesoderm (fig. 5, Pl. 2; fig. 1, Pl. 3), and in transverse section there is a slightly higher mitotic rate towards



TEXT-FIG. 5.

Longitudinal sections of a  $9\frac{1}{2}$ -mm. embryo. A, Sagittal section.

C, cartilage; Me, mesoderm and blood-vessels engulfed by anterior wall outpushings. Other lettering as before. B, Diagram of parasagittal section passing through one lateral lobe. Brain tissue, irregular lines; nervous lobe, small circles; presumptive proximal lobe, fine stippling; presumptive distal lobe, coarse stippling; lateral lobe, solid black.

the sides of the broad hypophysial cavity. The dorsal horns are prevented by the postoptic lamina from turning with the rest of the pouch tissue; they continue to grow actively both laterally and dorsally but do not meet above the neural stalk in the mouse.

Embryos of about 10 mm. often show short outpushings from the hypophysial cavity into the anterior and even the posterior thickening. They are finger-shaped, lined by a low columnar epithelium and with many mitoses in their walls. In these and later embryos occur small round or oval isolated cavities, possibly derived from the above (fig. 6, Pl. 3).

The lateral lobes appear in about 7-mm. embryos as two small lateral protuberances, independent of each other and of the

hypophysial stalk; they form in the same manner as the wall thickenings and as they grow forwards very few mitoses are to be found in their substance (fig. 7, Pl. 2). Cells push out at their tips below and to the sides but particularly upwards and forwards towards the brain floor; at the same time their bases enlarge and broaden to form a transverse ridge, continuous both with the anterior wall thickening and the hypophysial stalk. In transverse section the lobes in the 10-mm. embryo show as two lateral groups of cells (fig. 5, Pl. 2) with the nuclei arranged to surround irregularly an inner core of cytoplasm. In longitudinal sections they show on either side of the mid-line as sheets extending from the lower end of the pouch tissue towards the brain floor (Text-fig. 5 B), against which they meet to fuse and spread out by the 11-mm. embryo.

The earliest blood-supply of the future distal lobe of the adult gland (Text-fig. 1 c) is also accomplished by these growth movements. As the lateral lobes and the outpushings from the lower anterior wall develop they grow round and enclose the loose mesoderm of this region with its contained capillaries (Text-fig. 5 A). The latter in turn come to penetrate first the looser and then the denser parts of the neighbouring gland walls, often enlarging into little sinuses but generally containing no blood. The loosening of the tissue has now spread up the outer border and as this grows laterally it pushes out short blunt processes which similarly engulf the invaded mesoderm (fig. 1, Pl. 3). The nervous lobe and the future proximal lobe are supplied by peripheral capillaries which only later grow into their substance.

The establishment of the nervous lobe is now complete and the dorsal horns, lying close to the postoptic lamina, are constricting its root to form the neural stalk. In the part of the lobe distal to the brain the nuclei begin to round themselves off and move apart, in a manner comparable to that seen in the pouch walls, and as a result the lobe behind the dorsal horns begins to expand. Growth in size of the lobe is slow compared with that of the glandular regions and appears to be satisfactorily accounted for by the above process, along with a low but continuous mitotic activity.

In the 7-mm. embryo the neuro-ectodermal union was still

represented by a median mesoderm-free area but about 8 mm. isolated mesoderm cells begin to penetrate into the mid-line. Ill-defined patches free of mesoderm remain, however, usually one to either side, and can be distinguished in the 10-mm. embryo just behind the dorsal horns. It is in this general region that in some 10-mm. and nearly all 11-mm. embryos there appear small eruptions of cells from the presumptive proximal lobe into the nervous lobe, though whether they actually pass through these mesoderm-free patches or not cannot be said with certainty. At first these cells project into the nervous lobe as short finger-shaped processes whose darker cytoplasm makes them easily distinguishable; later their ends may expand and it becomes difficult to distinguish the two tissues (figs. 1-5, Pl. 3). So again it cannot be said with certainty whether actual cell migration is taking place.

Stage 5, fifteen to sixteen days old, 11 to 16 mm. in length, covering the establishment of the tuberal lobe, the appearance of fibres in the nervous lobe and of differentiation in the presumptive distal lobe.

Near the mid-line a relative flattening of the gland is becoming noticeable, doubtless the result of pressure from the brain above and the cartilage below, but laterally the great increase in breadth continues. The roots of the lateral lobes become incorporated in the outpushings from the anterior wall and a continuous shelf of tissue develops across the mid-line, separated from the wall of the hypophysial cavity above by a broken and irregular layer of enclosed mesoderm (Text-fig. 5). This layer can still be recognized in the adult as a region of concentration of numerous capillaries which are continuous from the distal lobe through the tuberal lobe on to the base of the brain. The incorporation of the lateral lobe roots is complete and no recognizable bands of tissue have been found in this region in later development (Baumgartner, 1916). Laterally this shelf appearance dies away since the sides of the gland are formed by lateral growth. The dorsal horns are bent well backwards, fusing gradually with the dorsal surface of the glandular tissue as it grows up on either side of the nervous lobe.

The individual cells of the presumptive distal lobe are losing



their embryonal appearance by about the 15-mm. embryo. Their cytoplasm becomes denser and stains more distinctly with haemalum and the difference between the close set cells of the original pouch wall and the looser outgrowing tissue disappears. The cells too become divided up by the end of the stage into groups bounded by connective tissue and with many large capillaries, but the latter are still mainly empty. Differentiation of the gland-cells may have begun but chromophil granules have not been demonstrated.

The tips of the lateral lobes, which have met and fused, completely lose their double nature to form the median tuberal lobe, a continuous mass of widely spaced cells passing halfway round the base of the stalk and well forwards below the brain (Text-fig. 6). By the end of the stage the tuberal lobe is relatively larger than at any other time; its cells are well dispersed through an open network of connective tissue with plenty of blood vessels, their cytoplasm definable and mitoses quite numerous.

The wall of the presumptive proximal lobe of the adult gland (Text-fig. 1 c) remains embryonal and its mitotic rate is high. The processes into the nervous lobe (fig. 1-5, Pl. 3) are prominent throughout the stage. They may be solid or tubular, may possess a short extension of the hypophysial cavity, may be central but more commonly lateral, may merely press against the connective tissue boundary of the nervous lobe or may penetrate its substance. On occasion it is again impossible to be sure whether or not the ends of the processes are actually contributing cells to the nervous lobe.

In the nervous lobe of the 12-mm. embryo fibres are beginning to appear in the neural stalk, causing the nuclei to concentrate round the diminishing central cavity, and by 14-mm. fibre formation has appeared in the end of the nervous lobe adjoining the neural stalk and is spreading peripherally. By the end of the stage the bulk of the cells in the lobe also are becoming concentrated around its cavity, with fibres and a few cells to the outside, but the nuclei are still rounded and embryonal. The mitotic rate is low but appreciable.

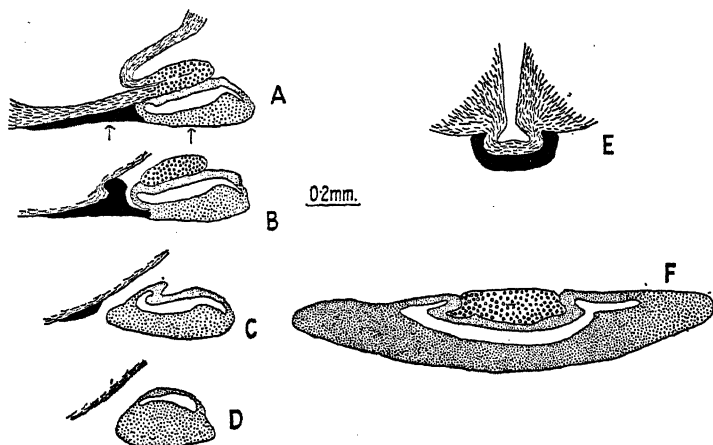
Stage 6, seventeen to eighteen days old, 16 to 22 mm. in length, covering the continued differentiation of the various lobes.

In the distal lobe a few acidophil cells, mainly lateral, show clearly at 18 mm. by Mallory's (1936) method after Bouin fixation, and their granules are so scanty that their first appearance seems unlikely to be significantly earlier. Indubitable basophils show at 22 mm. by iron haematoxylin and Mallory (Kerr, 1943) as larger cells staining a homogeneous pale blue. The capillaries of the distal lobe have been largely empty up to about 17-mm. embryos but by 19 mm. are beginning to contain more blood-cells and by 22 mm. are typically as full as in the adult. The change is both rapid and striking, so that even if the secretory products of the gland cells are elaborated before this time—without raising the question of what relation such products bear to the chromophil granules—it seems unlikely that they are put into circulation. By 20 mm. and over there is generally a sharp drop in mitotic activity. Apparently this is not due to the progress of differentiation, as it has increased again a few days after birth, and it may be due to approaching birth changes. The small isolated cavities mentioned in stage 4 have occurred in diminishing numbers in later embryos and are still occasionally to be found. Presumably many are obliterated during development but those which remain may possibly be the origin of the ciliated cysts found in some adult glands. The hypophysial stalk still persists to some degree in a proportion of embryos, sometimes as a long or short strand of cells and occasionally as a definite tube-like structure.

In about the 20-mm. embryo the proximal lobe begins to differentiate. Certain of its cells darken and elongate, extending often through the thickness of the lobe; the remainder become vacuolated in a characteristic way. With these changes the processes into the nervous lobe disappear and have not been seen later. The proximal lobe in the adult forms most of the tissue on the nervous lobe side of the hypophysial cavity, but distal lobe tissue extends around the edges of the cavity to meet it. The tuberal lobe becomes compressed against the brain, tapering away in front to a median point and behind broadening and merging into the anterior edge of the distal lobe. It is never associated with the proximal lobe (Text-fig. 6).

In the nervous component the neural stalk cavity partially

closes about 21 mm. and that of the nervous lobe itself usually before birth, but the end of the former nearer to the brain persists. As closure occurs the rounded embryonal nuclei around the cavity are becoming smaller and denser and more homogeneously distributed amongst the fibres. So histological differ-



TEXT-FIG. 6.

Diagrams to show the relations and relative sizes of the different regions of the pituitary gland in embryos of about 20 mm. A, Sagittal section. The arrows indicate the lines of cutting of the transverse sections E and F. B to D, Parasagittal sections from the same series as A, chosen at intervals between the mid-line and the edge of the hypophysial cavity. E, Transverse section through the tuberal lobe and the brain floor. F, Transverse section through the pituitary gland at its broadest part. Tuberal lobe, solid black. Other conventions as before.

entiation of both nervous and proximal lobes also begins very late in development, and it is difficult to believe that either could normally be functional earlier than the last day or so of pregnancy.

#### DISCUSSION.

The first stage in the development of the pituitary is taken as the attachment of stomodaeal roof and brain floor over a small area in front of the oral plate. This condition is apparently becoming established in 6-somite embryos and has become definite

by 10-somites, at a time when the stomodaeal roof is still flat and the brain open in front. The general closeness of brain and mouth in this region was first emphasized by Minot (1897), but the nature of the posterior attachment is not clear. In some early mouse embryos a genuine cytoplasmic fusion seems to be established whilst in others there appears to be no more than a close contiguity. In the embryo of the armadillo (Oldham, 1941) a basement membrane persists between the tissues and from appearance in dead mouse embryos this may well be the condition here also. Several functions may tentatively be ascribed to this neuro-ectodermal union. Its time of appearance makes an association with the induction of the hypophysial plate one possibility, later it may act as a point of attachment around which the plate can reorganize itself into a pouch, finally it may be concerned in the induction of the nervous lobe and act as a guide to the pouch in its early relations with this lobe. Lack or removal of either the neural or ectodermal tissue at the point of union should presumably lead therefore to the absence or abnormal development of the other component. There is supporting experimental evidence for this (e.g. Hilleman, 1943), but on the other hand Holt (1921) has described a normal nervous lobe in a pig embryo with no trace of a buccal component.

The delimitation of the hypophysial plate and the appearance of Rathke's pouch follow rapidly and at first simultaneously. The former is brought about not by a localized thickening of the ectoderm but by the retention in the hypophysial region of the primitive thickened condition of the earlier stomodaeal roof, thrown into relief by surrounding attenuation. The formation and closure of Rathke's pouch has been explained as a more or less automatic result of cranial flexure, invasion of mesoderm and other growth changes in the head region, rather than as an active pouch formation on the part of the hypophysial tissue itself (summarized in Gilbert, 1934; Kingsbury and Roemer, 1940). Partly this is a reaction against the misleading view that the pituitary is formed by one pouch from the stomodaeal roof growing up to meet a second pouch from the brain, but in the mouse it seems that such a mechanical interpretation is not entirely satisfactory. In early stages the posterior edge of the

hypophysial plate, i.e. that part behind the neuro-ectodermal union, is bent downwards to maintain attachment to the endoderm during the rapid early growth of the plica and spinal cord, the associated invasion of the mid-line by mesoderm being incidental. The anterior edge is correspondingly bent down by growth of the forebrain and increased cranial flexure. In sagittal section this appears sufficient to explain the establishment of the pouch, but laterally there is a simultaneous closing in of the edges of the hypophysial tissue surrounded only by loose embryonal mesoderm. The concentrations of mesoderm in the maxillary processes are too far out on either side to influence this process. In later stages the actual closure of the pouch, in which the lips bend inwards to meet from all sides, occurs not only before cartilage formation but before the organization of the prechondral nuclei which precedes it, so that appearances in the mouse lend no support to the supposition that closure is significantly affected by external forces. A better statement of the process might be that continual reorganization of the originally flattened hypophysial plate and a higher mitotic rate than the adjoining ectoderm and endoderm play as essential a part in pouch formation and closure as do external growth factors, that the growth of the pouch by its own activity keeps pace with the increasing gap between brain and gut, and that it is necessary to distinguish between growth changes in the head which affect pouch formation and those with which it is merely synchronized.

The enlargement and change in shape of the gland after pouch closure are conditioned by a number of factors, internal and external, varying in their effect upon the different regions. There is the underlying cartilage which, lacking a sella turcica, presents a ventral obstruction to gland enlargement matched above by a partial obstruction from the brain floor. Then the formation of the lateral lobes and the outpushings of the anterior pouch wall turn the long axis of the gland backwards and upwards, a reorientation most simply followed by its effect upon the hypophysial cavity, while at the same time the glandular tissue is extending laterally where its expansion is unimpeded. The growth of the presumptive distal lobe consists first in the loosening of the

primitive pouch wall, and the moving apart and rounding off of the nuclei, followed by the organization of the cells into blunt irregular processes. These invade the adjacent mesoderm and engulf it as a series of little intercommunicating islands with their pre-existing capillaries, which in turn form the basis of the connective tissue framework and the blood supply of the adult gland. The presumptive proximal lobe retains the primitive closely set condition of its wall until late in development.

There is no agreement upon the formation and growth of the nervous lobe in mammals. In the mouse it develops from the base of the premammillary lamina by a process not previously described. The whole brain wall in this region is at first thin, with mitoses scattered equally throughout, then mitoses become fewer in the lamina and more numerous to either side so that the lamina becomes distinguishable in transverse section as a curved area of roughly homogeneous thickness between two rapidly thickening regions of the brain wall. The lowest part of the lamina, just above the neuro-ectodermal union, now deepens to form a gutter; gradually the edges of the gutter meet, fuse, and separate off to form a diverticulum. This is the first stage in the development of the nervous lobe. At the same time as fusion commences at the lower end of the gutter its upper end becomes sharply marked off from the ordinary premammillary lamina above, and fusion of the gutter edges continues in an upward direction until only a small opening remains connecting the nervous lobe cavity with that of the brain. It is essential to follow this process in sections cut transversely to the lamina for in longitudinal views it is barely detectable. As regards the other theories mentioned in the Introduction, low power appearances do suggest that the brain floor is being pushed upwards by the pouch to form the nervous lobe (Mihalkovics, 1875), but between floor and pouch a small space is developing just at the time and place that pressure would have to be exerted. Again, mitoses are still quite common in the postoptic lamina; but this hardly supports Hochstetter (1924), since once the gutter walls have commenced to fuse the upper postoptic lamina is itself being formed from the gutter wall. Moreover the neuro-ectodermal union would probably prevent the implied independent

movement of this tissue over the top of the pouch. The more general movements listed by Gilbert (1934) in the cat are harder to assess. They may apply to the mouse in some degree apart from the formation of the gutter, but in detail there are differences from the cat. Mitoses do not virtually disappear at the critical time from the infundibular region, indeed in the nervous lobe and the lower preamillary lamina they are to be found in small numbers after colchicine treatment throughout the whole of embryonal development. The difficulty she finds in explaining the later growth in size of the nervous lobe without the aid of intrinsic cell division appears to be disposed of in the mouse by the amount of tissue originally segregated, by the wider spacing of the nuclei which then develops, by the appearance of fibres, and by the low but constant mitotic activity. Certainly no indications to suggest a migration of cells into the lobe from the brain (Gilbert, 1934; Kingsbury and Roemer, 1940) have been seen.

The significance of the small processes from the presumptive proximal lobe into the nervous lobe is not apparent, and their relation to the mesoderm-free areas derived from the neuroectodermal union uncertain. Brahms (1932) mentions an example of what might be a similar phenomenon in a single cat embryo but explains it differently; Atwell (1918) at one stage in rabbit embryos saw processes in this position but apparently developed in the opposite direction, i.e. from the nervous into the proximal lobe. Certainly appearances suggest that on occasion a restricted cell migration may be taking place, but even this cannot be stated unequivocally.

Mitoses are at first almost exclusively confined to the free zone of cytoplasm on the inside of the pouch. After closure this zone becomes indistinct and disappears but the mitoses still occur mainly on the cavity border, though with decreasing predominance, throughout the whole of development. In the lateral lobes and the outpushings of the pouch walls mitoses are not at first in evidence, as if migrating cells did not divide, but later mitoses become common enough.

## SUMMARY.

1. A description is given of the development of the mouse pituitary, with particular reference to some obscure points in the general embryology of the mammalian gland.

2. The establishment of the neuro-ectodermal union of brain floor and stomodaeal roof precedes the appearance of Rathke's pouch; its importance and the effects of external and internal factors on pouch formation and closure are discussed. It is considered that the process cannot be entirely due to mechanical influences and differential growth.

3. The nervous lobe forms from a groove in the premammillary lamina which deepens and separates off. Later enlargement of the lobe is due to the reorganization of its cells, the appearance of fibres and a low but steady mitotic activity.

4. The establishment of the lateral lobes and the blood supply, the distribution of mitoses, the development of processes from the proximal into the nervous lobe and other points are recorded.

I am again in debt to Professor E. A. Spaul, D.Sc., for reading the manuscript and for his invaluable discussion of the interpretations involved.

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## EXPLANATION OF PLATES.

### LETTERING.

*Bv*, cavity of brain; *Bw*, brain wall; *Dh*, dorsal horn of ectodermal component; *Dl*, distal lobe; *Em*, enterostomial membrane (oral plate); *Fg*, foregut; *Hc*, hypophysial cavity; *Ll*, lateral lobe; *Nl*, nervous lobe; *Neu*, neuro-ectodermal union; *Oc*, optic chiasma; *Pp*, prechordal plate; *Pl*, proximal lobe; *Plp*, process of proximal lobe; *Pml*, premammillary lamina; *Pol*, postoptic lamina; *Rb*, roof of buccal cavity; *Rp*, Rathke's pouch; *Sr*, stomodaeal roof; *St*, stomodaeum. Head end is to the left in the case of all longitudinal sections.

### PLATE 1.

Fig. 1.—Sagittal section of head end of a six-somite embryo to show close relation of brain floor and stomodaeal roof.  $\times 250$ .

Fig. 2.—Vertical section (horizontal to long axis of embryo) through area of apparent fusion at neuro-ectodermal union, ten-somite embryo.  $\times 400$ .

Fig. 3.—Section to show upgrowth of hypophysial tissue to either side of brain floor, 4-mm. embryo.  $\times 200$ .

Fig. 4.—Transverse section of Rathke's pouch, 3-mm. embryo.  $\times 200$ .

Fig. 5.—Sagittal section of head end of 4-mm. embryo.  $\times 50$ .

## PLATE 2.

Fig. 1.—Transverse section through preammillary lamina just above the neuro-ectodermal union to show first appearance of nervous lobe gutter, 4-mm. embryo.  $\times 200$ .

Fig. 2.—Transverse section through upper part of nervous lobe gutter, 7-mm. embryo.  $\times 150$ .

Fig. 3.—Transverse section below last to show gutter walls meeting and fusing, the tip of one dorsal horn also shows, 7-mm. embryo.  $\times 150$ .

Fig. 4.—Transverse section through nervous lobe, with dorsal horns to either side,  $7\frac{1}{2}$ -mm. embryo.  $\times 150$ .

Fig. 5.—Transverse section through gland to show the two lateral lobes growing forwards on either side of the mid-line, 10-mm. embryo.  $\times 75$ .

Fig. 6.—Sagittal section of gland to show stalk, prechondral nuclei and wall thickenings,  $7\frac{1}{2}$ -mm. embryo.  $\times 112$ .

Fig. 7.—Parasagittal section from same series as last to show appearance of one lateral lobe.  $\times 300$ .

## PLATE 3.

Fig. 1.—Transverse section of anterior part of gland to show dorsal horns partly enveloping front of nervous lobe, 12-mm. embryo treated with colchicine.  $\times 75$ .

Fig. 2.—Section to show outpushing of cells from proximal lobe into nervous lobe, with no sharp distinction between the tissues, 12-mm. embryo.  $\times 300$ .

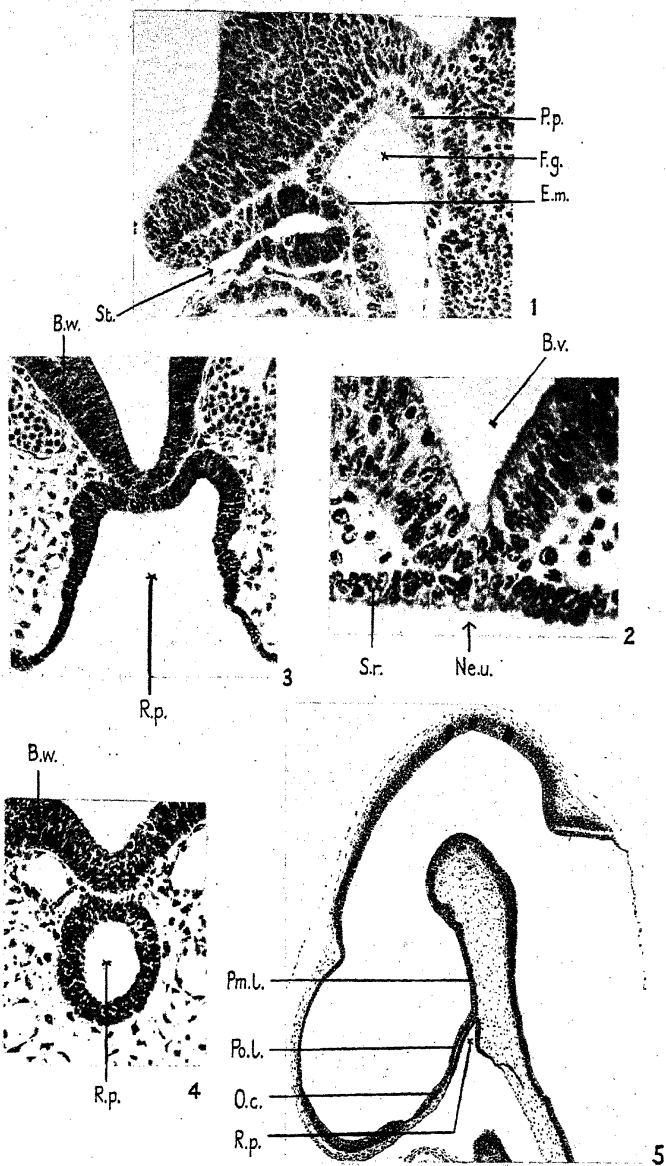
Fig. 3.—Section as last to show proximal lobe process containing a cavity, 15-mm. embryo.  $\times 300$ .

Fig. 4.—Section as last with proximal lobe process merely pressing against nervous lobe, 15-mm. embryo treated with colchicine.  $\times 300$ .

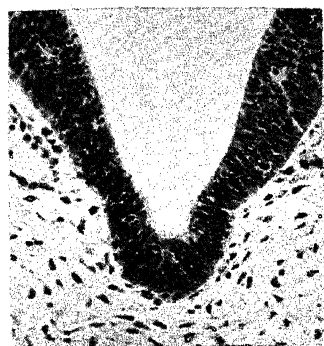
Fig. 5.—Section as last with process entering nervous lobe, 13-mm. embryo.  $\times 300$ .

Fig. 6.—Isolated cavity in distal lobe, 12-mm. embryo treated with colchicine.  $\times 400$ .

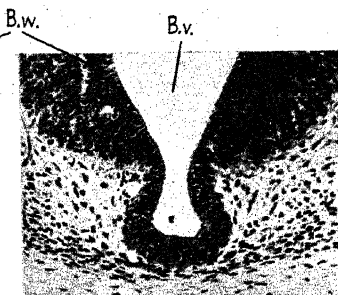




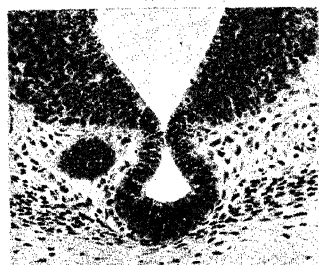




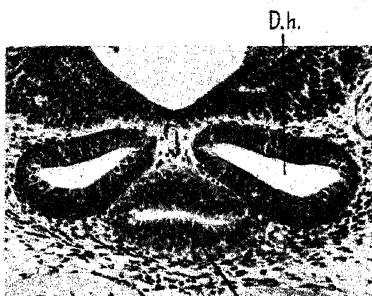
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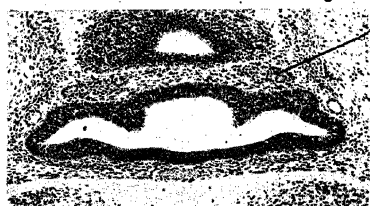
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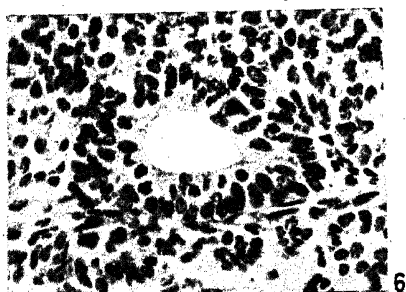
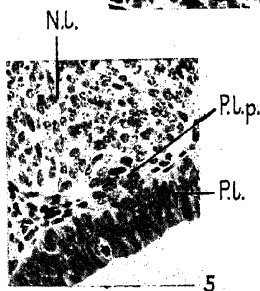
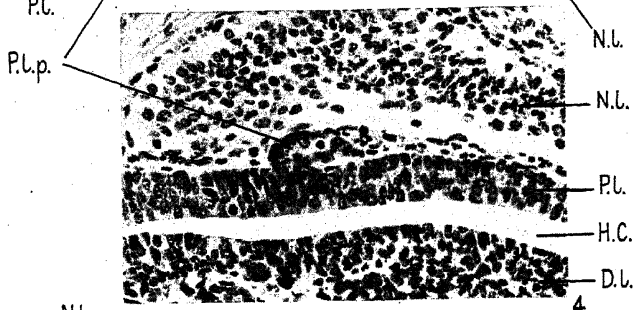
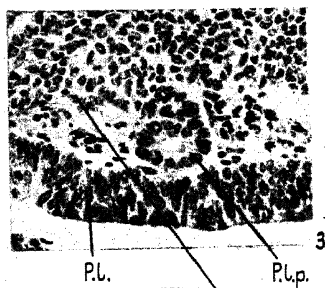
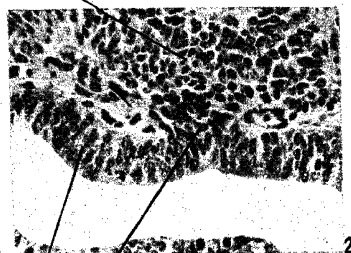
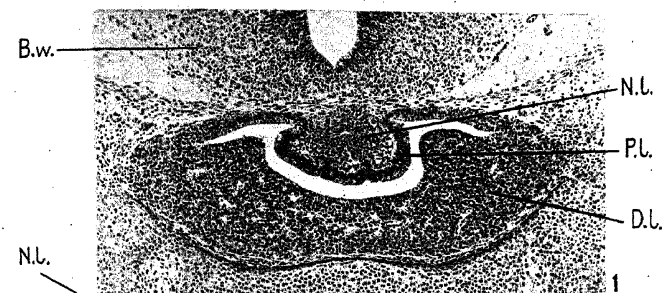


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# On the Origin of the Acrosome and the Behaviour of the Nebenkern in *Diestrammena* sp.

By

Chang-Chun Wu,

(Department of Biology, National University of Chekiang, Meitan,  
Kweichow, China).

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With Plates 4 to 6.

## INTRODUCTION.

IN descriptions of spermiogenesis, stress is laid on two factors. Of these, one is the origin and development of the acrosome; the other, the history of the mitochondria which later form the sheath of the sperm tail. Bowen (1922 e) described the relation of the idiosome and Golgi material to the acrosome, and indicated two ways in which the acrosome originates; one is the simple, or fused, type; the other is the compound, or multiple, type. Within these two types there is a further subdivision into vesicular and granular types, according to the kinds of acrosome formed, and into migratory and stationary types, according to the place of deposition. The compound, or multiple, type, as Bowen states, is very incompletely known and seems to be rare. The only known forms exhibiting this type occur in the Lepidoptera and in the Acridiidae among Orthoptera. In *Ceuthophilus* (a tettigoniid) Bowen (1922 d) found the origin of the acrosome to be of the simple and stationary type. The material employed for the present study, *Diestrammena* sp., is a genus closely related to *Ceuthophilus*. It is, however, quite different from the latter in respect to the origin of acrosome. In this respect it is very closely related to the type of the Acridiidae, to which family the grasshopper belongs. The history of mitochondria and Golgi material in the spermatocyte and in the early spermatid stages, and the later development of the acrosome in *Ceuthophilus*, were not recorded by Bowen. On the history of the Nebenkern and its later changes my observations agree

essentially with those made by him on Hemiptera and Lepidoptera (Bowen 1922 *a*, 1922 *b*, 1922 *c*), and I am in agreement with him in their interpretation.

#### MATERIAL AND METHODS.

The crickets—*Diestrammena* sp.—are obtainable in large numbers in early autumn in meadows in the vicinity of Hang-chow. For fixation of the testes, more than ten standard methods were tried: Champy-Kull, Mann-Kopsch, Kolatchew, Regaud, Gatenby's modified Flemming without acetic acid, Benda, Smith, Nawashin, Bouin, weak Flemming, &c. In Mann-Kopsch and Regaud preparations the cells showed much shrinkage, and the stages could not be followed satisfactorily; Gatenby's modified Flemming, Benda, and Kolatchew preparations all gave good general results, however. After fixation the material was put through the ordinary processes of dehydration and clearing and imbedded in paraffin wax. Sections were cut at 4 to 6 micra. Heidenhain's iron haematoxylin, gentian violet and Altmann's picric acid and acid fuchsin were especially useful for studying the origin of the acrosome. In the case of mature sperms, smear preparations were made from the vas deferens. They were fixed with Flemming's fluid and stained with gentian violet.

#### OBSERVATIONS.

In the early spermatocyte the nucleus is excentrically placed, the surrounding cytoplasmic layer being thicker on one side. In the thicker region of cytoplasm, and close to the nucleus, lies the idiosome, with the osmiophilic substance so aggregated as to give the appearance of a network. The chondriosomes are also situated in this thick cytoplasmic region, forming a nuclear 'cap'. This appears as a rather cloudy mass; the individual granules can scarcely be distinguished. As the spermatocyte begins to grow in size, the Golgi network breaks up into small pieces. Each piece has the form of a sickle, typically composed of an outer layer of dark osmiophilic substance and an inner lighter layer of osmiophobic substance. The former represents the Golgi material proper, while the latter represents the

idiosomic substance. At the same time the chondriosome mass also spreads out over the nucleus. When the spermatocyte has attained its full size, the Golgi bodies and chondriosomes distribute themselves evenly in the cytoplasm, while the nucleus itself takes up a more central position in the cell.

During the maturation divisions the chondriosomes assume the form of long threads and are distributed about the spindle; in general the threads lie parallel to the major axis of the latter. In telophase they are apparently divided in the middle (figs. 1 and 2, Pl. 4), so that each of the daughter cells receives about half the chondriosome material. So far as the chondriosomes are concerned, the second maturation division is a repetition of the changes observed in the first.

I was unable to follow the behaviour of the Golgi bodies during the maturation divisions; they are not visible from metaphase to telophase. When the spermatid is formed, however, they reappear as scattered sickle-shaped bodies (figs. 3 and 4, Pl. 4). The difficulty in detecting the Golgi material during these stages has also been reported by Gatenby (1918) in pulmonates.

In the early spermatids the Golgi bodies distribute themselves evenly around the nucleus (fig. 3, Pl. 4). To each fragment is attached a small round mass which stains red with acid fuchsin. It is clearly seen in Kolatchew preparations followed by Altmann's picric acid and acid fuchsin. The Golgi material is blackened by osmification, while the attached material stains red. Fig 3, Pl. 4, shows four of the Golgi bodies in that condition. The red-staining material is also observable in fully grown spermatocytes, but never found before the growth period.

This newly formed material is not identical with the idiosome substance, for the latter does not stain red with acid fuchsin. But whether the new material comes from the alteration of idiosome substance, or is secreted by the Golgi material anew, or arises as a result of both processes, is very difficult to ascertain. From the position it assumes, however, the formation of this material seems somewhat closely related to the idiosome substance. As the process continues, the Golgi bodies gradually aggregate between the nucleus and the Nebenkern. Although there is no direct evidence, this newly formed material appears

finally to fuse together to form a spherical acrosome (figs. 7, 8, and 9, Pl. 4), situated in the angle between the nucleus and Nebenkern. It stains homogeneously without showing any structure.

As soon as the Nebenkern begins to round up it becomes vacuolated, the vacuolation moving gradually towards the centre of the mass. Shortly after becoming spherical it is enclosed in a chromophobic envelope, through which pass numerous strands from the central chromophilic core (figs. 5 and 6, Pl. 4). Within the chromophilic core there are also numerous vacuoles which later enlarge, while the chromophilic material between them seems to decrease in mass, so that the whole structure now assumes a reticulated appearance. This is usually called the 'blackberry' stage. Eventually the vacuoles arrange themselves in definite layers and fuse together, giving rise to concentric clear spaces alternating with the thinner chromophilic material. The whole structure then resembles a bisected onion (fig. 5, Pl. 4). The onion-like Nebenkern now elongates somewhat (fig. 9, Pl. 4), while the peripheral, non-staining region becomes more sharply delimited (fig. 9, Pl. 4). At this stage the pattern of the Nebenkern shows bilateral symmetry; but its division into two equal halves does not occur until much later. The chromophilic material runs together and the chromophobic envelope correspondingly increases in volume. This is accompanied by the disappearance of a part of the chromophilic plate in the central core. Small globules now begin to appear in the lightly staining substance (figs. 10, 11, and 12, Pl. 5). This is the central substance. Later they join together to form beaded strands. While the chromophobic substance increases, the chromophilic substance gradually decreases in volume. Subsequently the Nebenkern divides into two equal halves, beginning from the two ends, while the chromophilic core shortens (fig. 11, Pl. 5). By the time the latter has entirely disappeared, the division of the Nebenkern is complete (fig. 12, Pl. 5).

The two halves of the Nebenkern now elongate rapidly, and the bulk of the granular central substance increases markedly. Later the Nebenkern spins out as two series of blebs (fig. 14, Pl. 5), which are the swollen vesicles of the

Nebenkern. Finally these vesicles disappear, and the material thus left forms the sheath of the sperm tail. Protoplasmic balls (figs. 15 and 16, Pl. 5) containing the chromatic granules, Golgi remnants, &c., migrate posteriorly along the tail and are eventually sloughed off.

On the opposite side of the Nebenkern (figs. 4, 7, 8, and 9, Pl. 4; fig. 13, Pl. 5) there are numerous granules which stain intensely with haematoxylin. The nature of these is unknown. Finally they slough off, together with the cytoplasmic remnant, along the tail.

The centrioles could not be clearly demonstrated in most stages. I think it is probable that the chromatic granules and the aggregated Golgi bodies render their observation difficult. In fig. 4, Pl. 4, the axial filament is demonstrated, but the centrioles cannot be made out. But in Bouin preparations (figs. 5 and 6, Pl. 4) in which the chromatic granules and the Golgi bodies are dissolved out by acetic acid, the two centrioles, from one of which emerges the axial filament, can be clearly seen. At first they are situated at the anterior pole of the nucleus, later migrating posteriorly around the nucleus to a position between nucleus and Nebenkern.

As the two halves of the Nebenkern elongate, the chromatin within the nucleus becomes spread out into a layer lining the nuclear membrane, while a few masses of indefinite form may also be seen in the nuclear cavity (fig. 15, Pl. 5). Later these masses dissolve, and the central cavity becomes quite clear. The cytoplasm in the region anterior to the nucleus gradually decreases in amount until the cell wall becomes closely applied to the nuclear membrane (fig. 15, Pl. 5).

The spherical acrosome now migrates to the anterior pole of the nucleus (figs. 15, 17, 18, 19, and 20, Pl. 5); while the Golgi remnants migrate posteriorly, together with the cytoplasmic remnant, along the tail. The acrosome then applies itself to the nuclear membrane (fig. 20, Pl. 5; fig. 21, Pl. 6).

The nucleus now begins to elongate in the direction of the major axis of the sperm. In the posterior region of the nucleus there is usually a clear round space in which lies a lightly staining mass (figs. 22 and 23, Pl. 6). This later diminishes in size and

disappears (fig. 24, Pl. 6). Two dark staining pieces then differentiate from the acrosomic material (fig. 22, Pl. 6) and later fuse at the anterior end. One piece, the perforatorium, lengthens out with a nodule at the apex. The bipartite nature of the acrosome can still be distinguished in figs. 24, 25, and 26, Pl. 6. The acrosome is really located somewhat laterally at the anterior end of the head. The nucleus also elongates and becomes spindle-shaped. Later the acrosome elongates still more and the demarcation of acrosome and sperm head (nucleus) is obliterated (figs. 28 and 29, Pl. 6). The centrioles now become more conspicuous. They lengthen out as two parallel rods, from one of which emerges the axial filament (fig. 29, Pl. 6). As the sperm becomes greatly elongated, the two rod-shaped centrioles also lengthen and fuse to form apparently a single rod, known as the centrosomal middle piece, behind the head (fig. 31 *a, b, c*, Pl. 6). In the nearly mature sperms the head and tail do not stain deeply with gentian violet, but the centrioles stain intensely, so as to form a conspicuous line across a sperm bundle in which the sperms are regularly arranged parallel to one another. The head elongates still more and, because of extreme chromaticity, its structures are no longer clearly visible. The acrosome now stains rather faintly (fig. 32, Pl. 6), and the nodules formerly situated at the apex disappear; at this stage the acrosome terminates in a very fine tip.

#### DISCUSSION.

1. Golgi Bodies and Acrosome.—From the results of numerous studies, notably those of Divaz, Bowen, Gatenby, &c., it seems certain that the acrosome owes its origin to the Golgi bodies. But the mode of its formation and later development is very variable. In most animals, for example, Hemiptera, Coleoptera, Mollusca, Amphibia, and Mammalia, it is regarded by Bowen as of the simple type, i.e. the Golgi bodies in the spermatids fuse to form a single acroblast from which the acrosome is secreted. In Lepidoptera and Acridiidae, Bowen described the origin of the acrosome as multiple. In the Acridiidae (*Rhomaleum* and *Dissosteria*) in particular, Bowen (1922 *d*) states that the Golgi bodies remain separate and never fuse to form a single massive acroblast. Later they migrate

back along the tail and are probably cast off with the protoplasmic remnant. The acrosome appears as an intensely staining globule in contact with the nuclear membrane, not far from the centrioles. Concerning the origin of the acrosome, Bowen (1922 *d*) made the following suggestion:

‘It seems probable, however, that from each one (Golgi body) is differentiated its small proportionate share, and by the deposition of many such parts the acrosome is gradually built up.’

He did not claim to have seen the acrosome actually formed by the Golgi bodies. In the present material the sudden appearance of the acrosome at a certain stage is much like that described above; but I was fortunately able to demonstrate several Golgi bodies bearing acrosomal material in the early stages.

In *Ceuthophilus* the origin of the acrosome was described by Bowen (1922 *d*) as of the simple type. The acroblast is a homogeneous globule and much like the acrosome of *Diestrammena*. But I found no evidence of vesicles being secreted from this globule, nor did I observe the casting off of any remnant. It is the globule itself that is applied to the anterior end of the nucleus and later forms the acrosome. Moreover, while the acrosome is formed the Golgi bodies still exist between the nucleus and the *Nebenkern* and do not clear away as Bowen's figures show (fig. 4, 1922 *d*). For this reason I regard the globule as the acrosome rather than as the acroblast. The later history of the acrosome has not been studied in detail by Bowen, either in *Tettigonidae* or *Aceridiidae*; but the small knob at the distal end and the duplex structure in the basal portion are very reminiscent of the present case.

In *Lepidoptera*, according to Gatenby (1917), all the Golgi bodies become swollen into vesicular spheres during the early spermatid stages, and these fuse to form the base of the acrosome. This agrees with my observations, but I was unable to make out the differentiation of the small dark-staining granules involved in the construction of the acrosome itself. I was, however, able to observe the swollen spheres in the later spermatocyte period. The casting off of the Golgi bodies after the completion of the acrosome was also seen (fig. 16, Pl. 5). The



process of deposition of the acrosome material by each Golgi body was not observed, nor could the differentiation of the acrosomal granule and the clear vesicle (Bowen 1922 *d*) be demonstrated.

Doncaster and Cannon (1920) described the 'acroblast' in *Pediculus* as a spherical, evenly staining body, which later becomes pressed against the nuclear membrane, spreads out over one side of it like a small cap, and then grows forward over the nucleus until its anterior end projects in front of the nucleus. They were unable to determine the origin or nature of the 'acroblast' with certainty, but they suspected that it belongs to the Golgi apparatus. Later Cannon (1922) described the 'acroblast' as arising from two or three scattered granules which he thought to be the true Golgi bodies. This spherical 'acroblast' may well be comparable with the acrosome in the present material. I think it is much better to call it the acrosome or proacrosome, since it forms the acrosome directly at the front of the sperm. According to Bowen the acroblast represents the fused Golgi bodies, from which the acrosome is secreted, and which do not directly form the acrosome.

From the observations of many workers (Gatenby and Woodger (1921), Oliver (1913), Papanicolaou and Stockard (1918), Bowen (1920, 1922 *a*, 1922 *e*, 1924, &c.)), and also from the present study, I am inclined to believe that the origin of the acrosome (or proacrosome of some authors) is more closely related to the idiosome or archoplasm, with which the Golgi material is usually associated. In many cases the acrosome material first differentiates within the idiosome; and in Bowen's figure the acrosomal vesicle also arises from the chromophobic material which he took to be the idiosome substance. Whether the Golgi material (osmiophilic part) acts as a centre of activity or contributes materially to the acrosome we cannot decide at present.

In *Gelastocoris* Payne (1929) described the acrosome arising from the fusion of a large number of small proidiosomal spheres present throughout the later period of spermatocyte development. The origin of these spheres was doubtful but he interpreted them, without direct evidence, as arising from, or

having some connexion with, the Golgi bodies. As fusion progresses the Golgi bodies aggregate around their peripheries. Whether the Golgi bodies contribute anything to the idiosome at this time he was unable to say. Voinov's 'appareil sphérolaire' is probably of the same nature as Payne's proidiosomal granules.

2. Chondriosomes.—In my material the early history and behaviour of the chondriosomes during the maturation divisions agree with those of many other forms (Bowen (1920), Payne (1916, 1929), Shaffer (1917), Pollister (1930), Gatenby (1922), &c.). As in the Hemiptera (Bowen 1922 *a*), the chondriosomes condense to form the Nebenkern and later form the sheath of the tail. The various patterns assumed by the different stages of the Nebenkern have been carefully studied by Bowen (1922 *b*) and closely agree with those observed in the present material. The chromophilic material gives one the impression that it is present in the form of a plate rather than in a spireme, as suggested by Gatenby (1917, 1931).

#### SUMMARY.

1. In the early spermatocyte of *Diestrammena* the Golgi material and the chondriosomes are aggregated on one side of the nucleus. During the growth period both spread out around the nucleus and become evenly scattered through the cytoplasm.

2. During the maturation divisions the chondriosomes, in the form of threads, surround the mitotic figure as a mantle and are distributed equally to the daughter cells during telophase. During these stages Golgi bodies could not be demonstrated by any of the methods used.

3. The acrosome is of multiple origin. It is formed separately from the chromophobic material of the Golgi bodies, which never fuse together to form a single acroblast, as is frequently observed in other forms. After the acrosome is complete the Golgi remnants migrate posteriorly and slough off along the tail with the cytoplasmic remnant.

4. The acrosome sphere migrates to the anterior pole and presses on the nuclear membrane. Within this sphere two

portions of chromatic substance are formed; these fuse and elongate to form the front piece of the sperm.

5. As spermiogenesis goes on, the chromatin within the nucleus forms a peripheral layer inside the nuclear membrane. Finally the nucleus elongates to form the head of the sperm.

6. The chondriosomes condense to form the *Nebenkern*. This then differentiates into chromophilic and chromophobic substance. The former appears as a plate embedded in the latter and gradually decreases in amount. At the same time the central substance appears within the chromophobic substance. When the chromophilic substance has completely disappeared, the *Nebenkern* divides into two equal parts. It then spins out as two series of blebs. Eventually the blebs disappear and the materials thus left twist around each other to form the sheath of the tail.

7. On the anterior side of the nucleus there exist some granules. Their origin and nature are doubtful. They resemble the fat droplets in the spermatid of Hemiptera. Ultimately they are sloughed off along the tail together with the cytoplasmic remnant.

The writer wishes to express his thanks to Professor S. Hsü, under whose supervision this work has been carried out. He is also indebted to Professor S. Pai for his valuable suggestions and criticisms in reading over the manuscript.

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## EXPLANATION OF PLATES 4 TO 6.

All the figures were outlined as far as possible with a camera lucida and the details filled in freehand. Fig. 32 is at a magnification of about 1,450 diameters; all the others about 3,750 diameters.

## LETTERING.

*ac*, acrosome; *blnb*, blebs of Nebenkern; *c*, centriole; *Cch*, condensed chondriosomes; *cchr*, condensed chromosomes; *CGb*, group of Golgi bodies; *ch*, chondriosomes; *chrc*, chromophilic core; *chre*, chromophobic envelope; *chrg*, chromatic granules; *chrm*, mass of chromatin; *chrpl*, chromatin applied to nuclear membrane; *chrr*, chromatic remnants; *Csub*, central substance; *cm*, cell membrane; *cyt*, cytoplasm; *cytr*, remnant of cytoplasm; *Gb*, Golgi bodies; *Gbr*, remnant of Golgi body; *mb*, mid-bodies of spindle; *mp*, centrosomal middle-piece; *n*, nucleus; *Nb*, Nebenkern; *shspt*, sheath of sperm tail; *spf*, spindle fibres (the pointer is slightly too long and points to a chondriosome); *spr*, spindle remnant; *vac*, vacuoles in Nebenkern.

## PLATE 1.

Fig. 1.—Telophase of the first maturation division, showing the division of the chondriosomes. (Gatenby-haematoxylin.)

Fig. 2.—Newly formed spermatid, spindle remnant still present, chondriosomes beginning to condense. (Gatenby-haematoxylin.)

Fig. 3.—Spermatid; four Golgi bodies with acrosomal material on one side. (Kolatchew-fuchsin.)

Fig. 4.—Spermatid; showing the posterior migration of the centriole (from the position of axial filament). (Gatenby-haematoxylin.)

Fig. 5.—Spermatid; showing the two centrioles, from one of which emerge the axial filaments; the Nebenkern differentiated into chromophobic envelope and chromophilic central core. (Bouin-haematoxylin.)

Fig. 6.—Spermatid; vacuolation of the Nebenkern more advanced; the centrioles and axial filament also demonstrated. (Bouin-haematoxylin.)

Fig. 7.—Spermatid; acrosome appearing in the angle between Nebenkern and nucleus. (Kolatchew-fuchsin.)

Fig. 8.—Spermatid; Nebenkern assuming the form of a bisected onion bulb; also demonstrates the acrosome. (Kolatchew-fuchsin.)

Fig. 9.—Spermatid; showing the elongation of the Nebenkern; also demonstrates acrosome and chromatic granules. (Benda-haematoxylin.)

## PLATE 2.

Fig. 10.—Spermatid; showing the diminution of chromophilic central core and appearance of the central substance (nucleus not shown). (Gatenby-haematoxylin.)

Fig. 11.—Later stage than Fig. 10. (Gatenby-haematoxylin.)

Fig. 12.—Spermatid; the chromophilic substance has completely disappeared and the Nebenkern is divided into two halves. (Benda-haematoxylin.)

Fig. 13.—Spermatid; the central substance more increased and the Nebenkern more elongated. (Champy-Kull-haematoxylin.)

Fig. 14.—Later spermatid; the Nebenkern spinning out as two series of blebs. (Gatenby-haematoxylin.)

Fig. 15.—Later spermatid; showing one series of Nebenkern blebs; chromatin within the nucleus aggregated into a peripheral layer. (Gatenby-haematoxylin.)

Fig. 16.—Cytoplasmic remnant in which are embedded the Golgi remnant and the chromatic granules. (Gatenby-haematoxylin.)

Fig. 17.—Later spermatid; the acrosome beginning to migrate to the anterior pole; Golgi remnant and chromatic granules sloughed off posteriorly. (Champy-Kull-haematoxylin.)

Fig. 18.—Later stage than Fig. 17. (Champy-Kull-haematoxylin.)

Fig. 19.—Later stage than Fig. 18. (Kolatchew-fuchsin.)

Fig. 20.—Later spermatid; the acrosome pressed to the anterior pole of the nucleus. (Kolatchew-fuchsin.)

### PLATE 3.

Fig. 21.—Later stage than Fig. 20. (Kolatchew-haematoxylin.)

Figs. 22-7.—Elongation of the nucleus and later development of the acrosome. (Champy-Kull-haematoxylin.)

Fig. 28.—Further elongation of the nucleus and acrosome. (Champy-Kull-haematoxylin.)

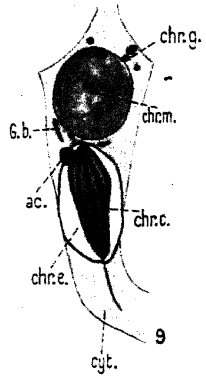
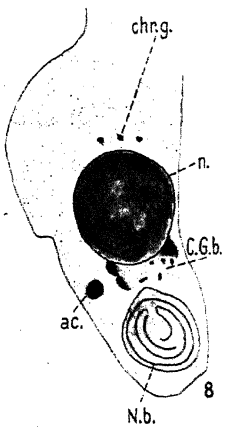
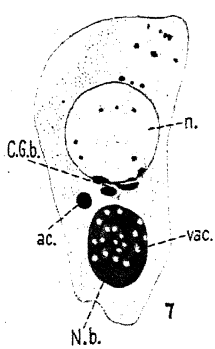
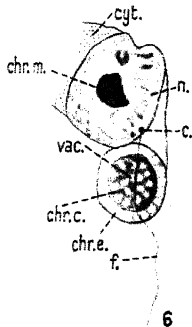
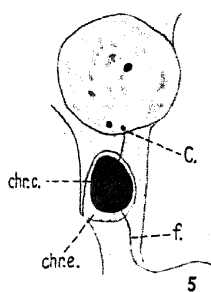
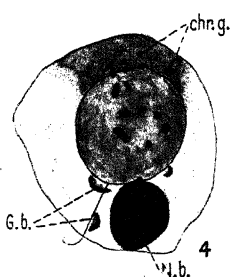
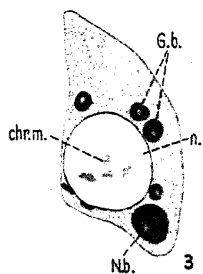
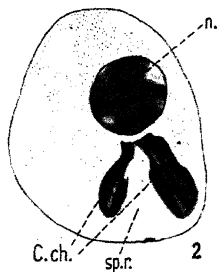
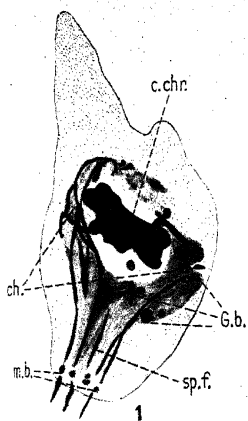
Fig. 29.—Nearly mature sperm; the centrioles as two rods from one of which emerges the axial filament. (Bouin-haematoxylin.)

Fig. 30.—Anterior portion of the acrosome, much elongated as compared with Fig. 29. (Champy-Kull-haematoxylin.)

Fig. 31 *a, b, c*.—Centrioles elongating and fusing to form the centrosomal middle piece. (Modified Flemming-Gentian violet.)

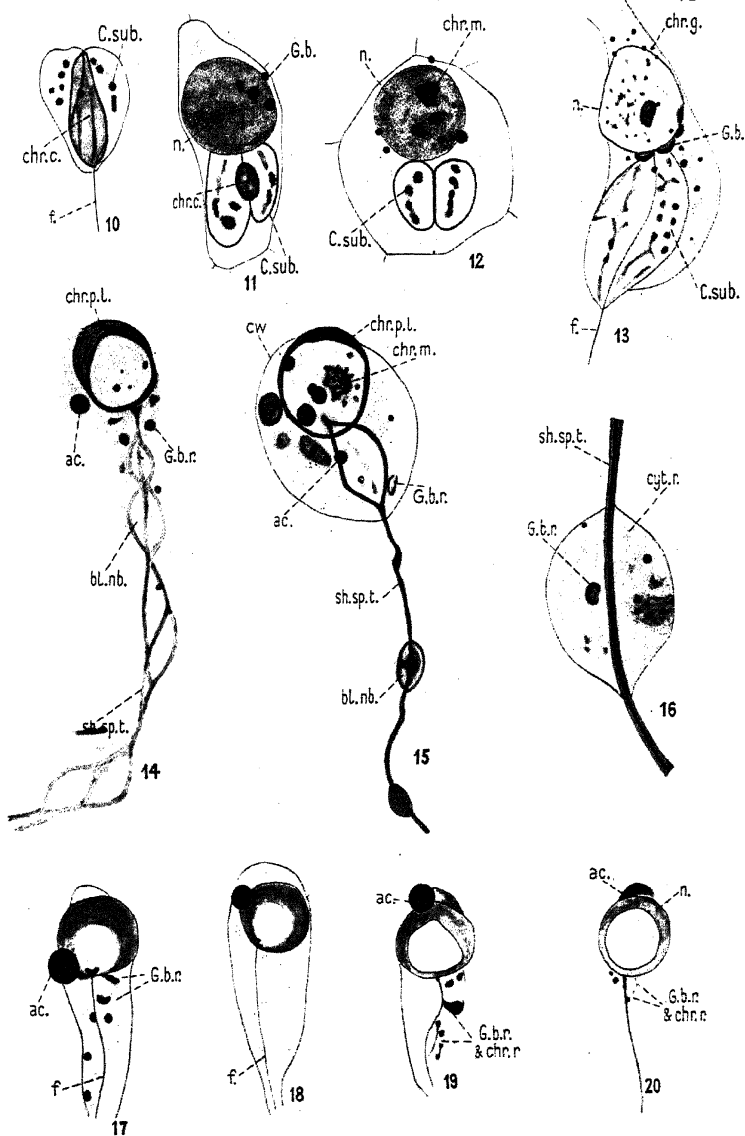
Fig. 32.—Mature sperm head from the vas deferens. (Smear preparation: Benda-Gentian violet.)













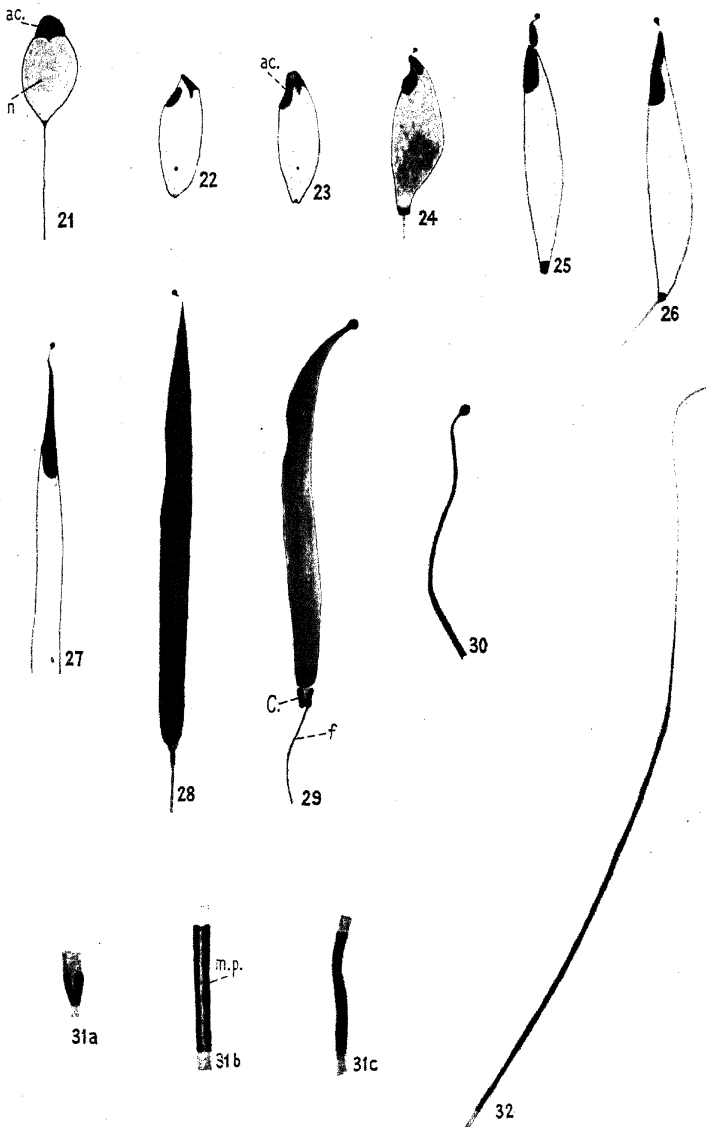
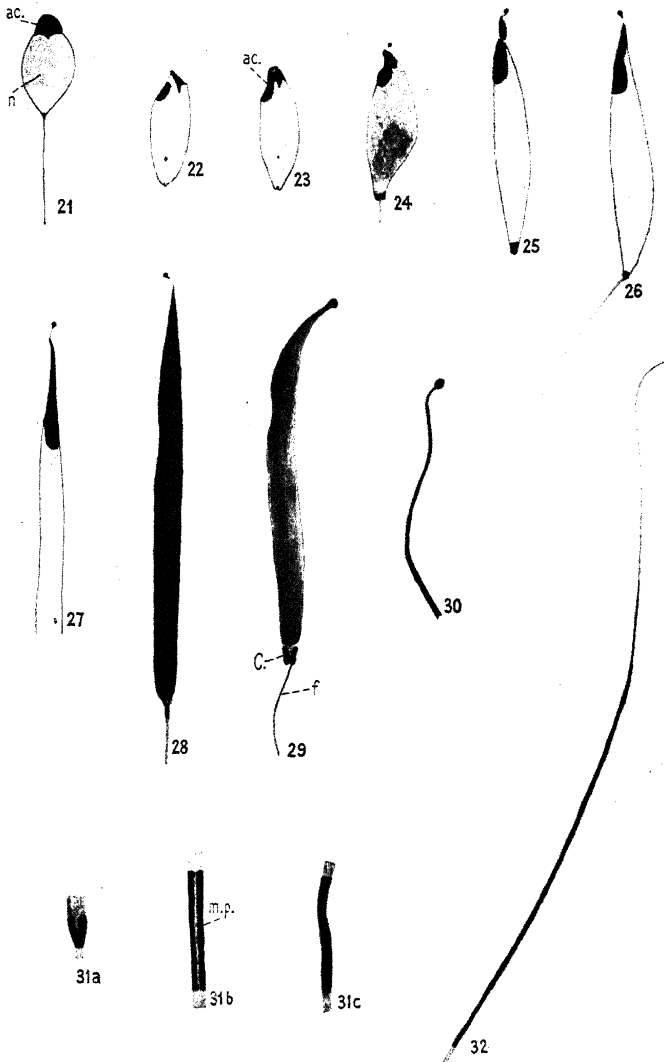




PLATE III





# Studies on the Structure, Development, and Physiology of the Nephridia of Oligochaeta.

## Part VII. The Enteronephric Type of Nephridial System in Earthworms belonging to three species of *Megascolex* Templeton and three species of *Travoscolides* Gates (*Megascolides* McCoy).

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With 4 Text-figures.

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### 1. INTRODUCTION.

IN Parts IV and V of this series of memoirs (3 and 4), I described the enteronephric type of nephridial system in four species of the genus *Megascolex*, and predicted that many, if not all, of the species of this genus would be found to possess an enteronephric system essentially similar to that of *Pheretima* (2). I have since been trying to obtain specimens of as many species of *Megascolex* as I can to examine their



nephridia and to ascertain whether they are enteronephric or not. The genus *Megascolex* comprises as many as fifty-six Indian species which are found mainly in Ceylon and the extreme south of the Indian Peninsula. Stephenson (8) has rightly remarked that 'Ceylon has the greater number of species and is the home of the genus par excellence'. I have been fortunate in securing specimens of another four species and find that three of them are enteronephric, while one is exonephric. Of the three enteronephric species, two show this type of excretory system in its best developed form in the genus *Megascolex*, a form which must have been the immediate precursor of the excretory system of *Pheretima* in evolution.

But the most interesting condition of the nephridial system has been found in *Travoscolides chengannures* in which the nephridia in the whole body are exclusively enteronephric. Although the enteronephric system has been described in the genera *Pheretima*, *Lampito*, *Woodwardiella*, *Tonoscolex*, and several species of *Megascolex*, it occurs side by side with the exonephric system in all these five genera. But the genus *Travoscolides* is unique in possessing only enteronephridia, so that one can truly say that the enteronephric system has reached its high-water mark in this genus—all the nephridia opening into the pharynx, oesophagus, and the intestine, there being no exonephridia at all in any part of the body.

The absence of the enteronephric system has already been recorded in *Megascolex templetonianus* (4) and I am now able to state that this type of nephridial system is also absent in one other species of *Megascolex*, i.e. *Megascolex caeruleus*. *Megascolex caeruleus* is further distinguished by possessing ventral phagocytic organs as have already been described by me in *Megascolex templetonianus* (3).

My best thanks are due to Dr. B. N. Chopra, officiating Director of the Zoological Survey of India, through whose courtesy I was able to obtain specimens of several species of *Megascolex*, to Mr. P. Kirtisinghe of the University of Ceylon who kindly sent me two fine specimens of *Megascolex*

caeruleus, and to Dr. N. K. Panikkar of the University of Madras who kindly sent me specimens of *Travoscolides chengannures* from Travancore.

## 2. THE ENTERONEPHRIC SYSTEM IN THREE SPECIES OF MEGASCOLEX.

### (a) *Megascolex campester* Steph.

This species was first described in 1915 by Stephenson (7) who gave a detailed account of the nephridia as follows: 'The nephridial system shows a combination of mega- and micronephridia. The micronephridia are very numerous and minute, on the inner surface of the body-wall; at the anterior end they are very noticeable on the dorsal wall of the pharynx and buccal cavity around the cerebral ganglion. Commencing from segment XX there are larger nephridia also; but in calling these meganephridia<sup>1</sup> I do not wish to imply that they are necessarily different in kind from the smaller micronephridia.<sup>1</sup> These larger nephridia are not, at first, present in all segments or always on both sides of the same segment; the series becomes more regular farther back. At the posterior end there is a difference; the number of the small micronephridia is still large, but the larger nephridia, much more opaque and obviously of far greater calibre, standing out distinctly on opening the worm and easily visible to the naked eye, are more numerous. There are usually two on each side in each segment and sometimes three; each is a small coil of a few turns or loops, without any connexion with a septum.<sup>1</sup>' This description of the nephridia was written at a time when the enteronephric system had not been discovered; Stephenson, therefore, did not suspect its existence in this species.

I have been fortunate in obtaining one of the three specimens originally examined by Stephenson and find that his description is only partially correct and that he has missed altogether the essential characters of the 'meganephridia'. Stephenson is right in saying that numerous minute micronephridia are present in almost all the segments of the worm; they are, in

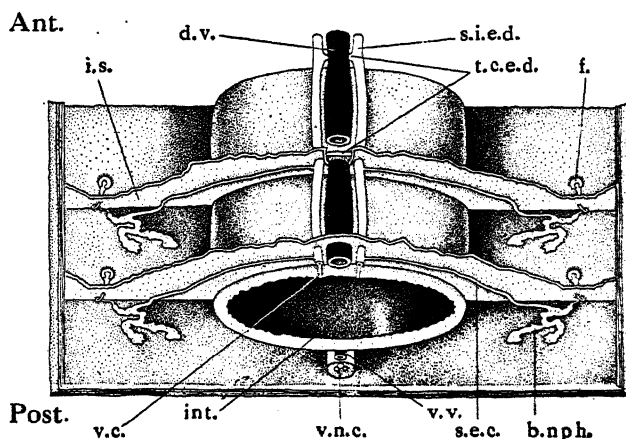
<sup>1</sup> The spaced words are mine.

fact, integumentary meronephridia attached to the parietes and open on the body-wall (exonephric), like the integumentary nephridia of *Pheretima*. But the larger nephridia (meganephridia) are actually different in kind from the micronephridia; they are really larger septal meronephridia with distinct pre-septal funnels and are enteronephric. Stephenson missed the funnels and also made a mistake in saying that these larger nephridia are 'without any connexion with the septum'. Further, Stephenson is not right in calling them 'meganephridia' although they are slightly larger than the integumentary nephridia. It is best to describe all the nephridia of this species as meronephridia.

On opening a worm by a mid-dorsal incision one immediately notices under a binocular dissecting microscope a pair of translucent longitudinal ducts, lying on the intestine, one on each side of the dorsal vessel, and running parallel to it; these are the lateral supra-intestinal excretory ducts (Text-fig. 1). This lateral position of the supra-intestinal excretory ducts is interesting as it represents a condition primitive to that of *Pheretima* in which these ducts always lie close together beneath the dorsal vessel. Apparently Stephenson could not observe these ducts, or if he did, he mistook them for blood-vessels, and did not mention them. The essential features of the septal meronephridia, and their ducts and openings into the intestinal lumen, are shown in Text-figs. 1, 2, and 3.

The septal nephridia begin from segment XX and continue right up to the anal segment of the worm. These nephridia are very few in number—two or three on each side in each segment as observed by Stephenson. Each nephridium (Text-fig. 2) has essentially the same structure as that of *Pheretima* but there are differences in details. The pre-septal funnel is rather stout; its upper and lower lips are more or less at the same level, and as they generally gape apart, the funnel presents a vase-shaped appearance. It is followed by a thick neck, which perforates the septum, and the following canal runs for a long distance behind the septum before entering the body of the nephridium. The body of the nephridium is comparable with that of *Pheretima*, consisting of a short straight lobe and

a long twisted loop, the latter extending beyond the base of the straight lobe towards the canal following the funnel. The straight lobe is  $530\mu$  in length, the twisted loop  $1,030\mu$  in length, while the terminal nephridial duct is exceedingly long and coiled



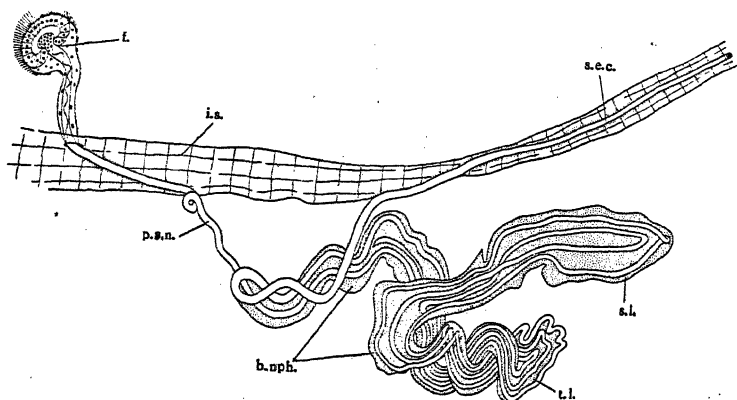
TEXT-FIG. 1.

A diagrammatic representation of the enteronephric excretory system of *Megasciolex campester* as seen in a dissection from the dorsal side. For the sake of clearness only one septal nephridium is shown on each side in a segment, and the integumentary nephridia are omitted altogether. *b.nph.*, body of the septal nephridium; *dv*, dorsal vessel; *f*, pre-septal funnel of septal nephridium; *int.*, lumen of the intestine; *is*, intersegmental septum; *sec*, septal excretory canal; *sied*, lateral supra-intestinal excretory duct; *tced*, transverse connexion between the two excretory ducts; *vc*, vertical canal from the lateral supra-intestinal excretory duct opening into the lumen of the intestine; *vnc*, ventral nerve-cord; *vv*, ventral vessel.

before it reaches the septum on which it runs to join the septal excretory canal. The septal excretory canal of each side runs dorsalward to open into the lateral supra-intestinal excretory duct of its own side.

The two supra-intestinal excretory ducts open separately into the lumen of the intestine, each by its own vertical canal in each segment, the openings being guarded by thick tubular sphincter muscles (Text-fig. 3 B). These openings, a pair in

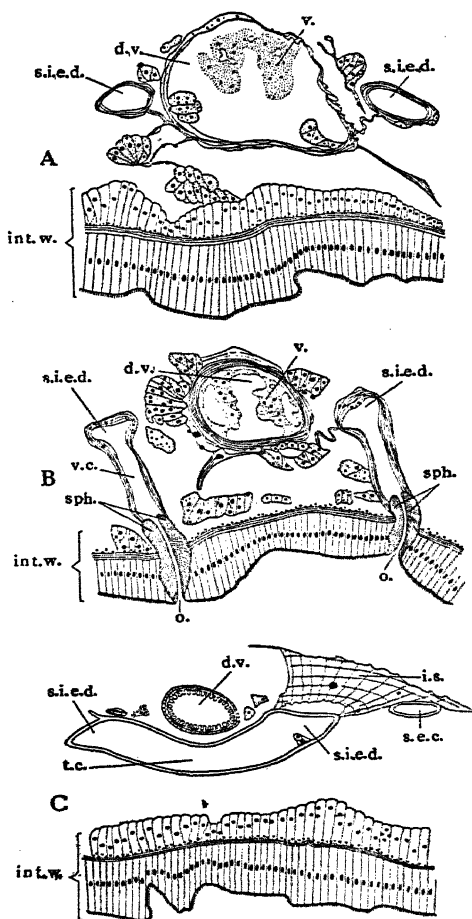
each segment, lie just behind each intersegmental septum, while along the anterior face of each septum the two supra-intestinal excretory ducts are connected with each other by a transverse cross-connexion running beneath the dorsal vessel



TEXT-FIG. 2.

A septal nephridium of *Megascycolx campester* with its terminal nephridial canal. *b.nph.*, body of the nephridium; *f.*, pre-septal funnel; *i.s.*, intersegmental septum; *p.s.n.*, post-septal part of the neck of the funnel; *sec.*, septal excretory canal; *sl.*, straight lobe; *tl.*, twisted loop. ( $\times$  cir. 71.)

(Text-figs. 1 and 3 c). The two lateral supra-intestinal excretory ducts and their segmental transverse connexions thus form a ladder-like arrangement on the dorsal surface of the intestine, as shown in Text-fig. 1. It may be mentioned here that the septal excretory canals and the supra-intestinal excretory ducts are large and prominent in this worm, particularly when we bear in mind the fact that there are only four to six septal nephridia in each segment. Further, the supra-intestinal ducts extend continuously throughout the region of the septal nephridia as in *Pheretima*, *Lampito*, and *Woodwardiella*, unlike the condition in *Tonoscolex* and several species of *Megascycolx* in which there are no continuous supra-intestinal ducts, and the septal excretory canals alone join together to open into the intestine in each segment.



TEXT-FIG. 3.

Three transverse sections of *Megascycolx campester* showing the disposition of the supra-intestinal ducts in various regions. A, through the middle of a segment. B, just behind an intersegmental septum. C, just in front of an intersegmental septum. *dv*, dorsal vessel; *intw*, wall of the intestine; *is*, intersegmental septum; *o*, opening of the vertical canal into the intestinal lumen; *sec*, septal excretory canal; *sied*, lateral supra-intestinal excretory duct; *sph*, sphincter muscle surrounding the opening of the vertical canal into the lumen of the intestine; *tc*, transverse canal connecting the two lateral supra-intestinal ducts; *v*, valves in the dorsal vessel; *vc*, vertical canal leading from the supra-intestinal duct to open into the intestinal lumen. ( $\times$  ca. 115.)

Other features worth recording in this species of *Megascolex* are the absence of pharyngeal and tufted nephridia in the pre-clitellar segments and the presence of scattered integumentary nephridia in all the segments of the worm.

(b) *Megascolex bifoveatus* Steph.

Stephenson (7) described the nephridia of this worm as follows: 'micronephridia few or absent on body-wall in front of clitellum, otherwise present throughout; at some point behind the clitellum larger nephridia appear, each consisting of a wavy or twisted tube, or of a number of coils, but of no great size; at first these are one on each side per segment, but towards the hinder end they are two or three on each side, some being attached to the anterior septum; these larger nephridia do not seem to be different in kind from the micronephridia.'<sup>1</sup>

This description of nephridia was written at the same time (1915) as that of *Megascolex campester*; Stephenson, therefore, could not suspect that in this species also the larger nephridia behind the clitellum are really enteronephric septal nephridia. In the post-clitellar segments both septal and integumentary nephridia are present. The septal nephridia are larger in size; they have long-necked pre-septal funnels leading into post-septal bodies of nephridia, the terminal nephridial ductules of which travel dorsally on the posterior faces of the septa and open into two lateral supra-intestinal excretory ducts, one on each side of the dorsal vessel, the ducts themselves opening into the intestinal lumen in each segment exactly as in *Megascolex campester*. The larger nephridia are, therefore, enteronephric and are actually different in kind from the micronephridia which are really exonephric integumentary nephridia. The septal nephridia (0.40 mm. long) are about two and a half times as long as the integumentary nephridia (0.16 mm. long). The number of septal nephridia towards the hinder end is five or six on each side and not two or three, as Stephenson describes. There are no pharyngeal

<sup>1</sup> The spaced words are mine.

nephridial tufts, nor are there any tufted nephridia in the pre-clitellar segments.

The nephridial system of this species strongly resembles that of *Megascolex campester* with its ladder-like arrangement of lateral supra-intestinal excretory ducts and their cross-connexions beneath the dorsal vessel. The differences are that there are no septal excretory canals, and the terminal nephridial canals travel individually on the septa all the way along and open directly into the lateral supra-intestinal ducts; further, the nephridia are much smaller in size and so are the lateral supra-intestinal excretory ducts. The nephridial system of this species is, therefore, primitive to that of *Megascolex campester*; in fact it gives the idea of being a miniature copy of the nephridial system of *Megascolex campester*.

It is interesting to note that both *Megascolex campester* and *Megascolex bifoveatus* with remarkably similar nephridial system occur in the same locality in Ceylon, i.e. Horton Plains, about 7,000 ft. above sea-level.

(c) *Megascolex cingulatus* (Schmarda).

This species was first described by Schmarda in 1861 and again by Beddard, Michaelsen, and Stephenson. Schmarda's original paper is not available to me, but I have consulted the papers of the other three authors and find that none of them has said even a word about the nephridia of this worm.

Although the single specimen available to me was not well preserved, I could easily make out, in the intestinal region of the worm, septal nephridia with pre-septal funnels and the slender septal excretory canal on each side running parallel to the commissural vessel. In sections the two septal excretory canals are seen to meet in the mid-dorsal line in each segment beneath the dorsal vessel and to open, through the typhlosole, into the lumen of the intestine in each intersegmental place, showing that the excretory system is enteronephric. There is no continuous supra-intestinal excretory duct, so that the excretory system is a separate entity in each segment and there is no connexion from segment to segment. The excretory



system, therefore, resembles that of *Megascolex cochiniensis* (3).

### 3. EXCLUSIVELY ENTERONEPHRIC EXCRETORY SYSTEM IN TRAVOSCOLIDES.

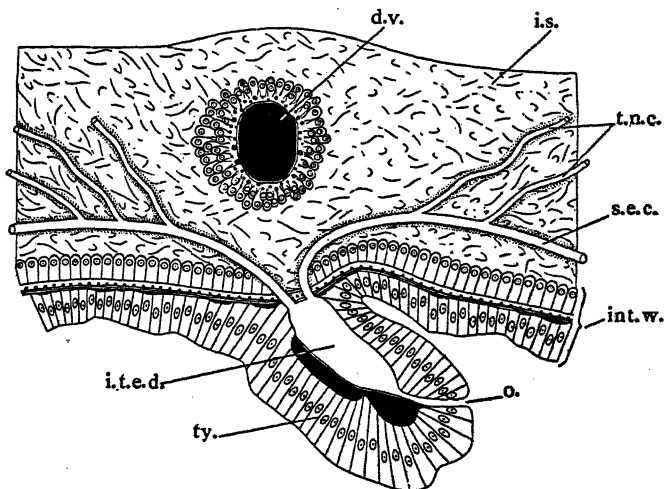
The species *Travoscolides chengannures* was instituted by Aiyer (1) in 1929 who placed it under the genus *Megascolides* which according to Stephenson already comprised seven species of Indian earthworms. Gates (6) has recently re-examined the eight Indian species of *Megascolides* and has classified them into three distinct genera, i.e. *Megascolides*, *Barogaster*, and *Travoscolides*. Under *Travoscolides* he includes four species, i.e. *chengannures*, *cochinensis*, *duodecimalis*, and *pilatus*, and makes *Travoscolides chengannures* as the genotype. Examination of the nephridia of three out of these four species convinces me that the separation of these species by Gates into the new genus *Travoscolides* is fully justified.

Aiyer (1) described the nephridial system of *Travoscolides chengannures* as follows: 'In the pre-clitellar region the nephridia are large "tufts", one pair per segment, at the sides of the oesophagus. From segment XIV onwards a large number of small micronephridia are arranged in each segment in a single transverse row close to the septum. There are no integumental nephridia in any part of the body.' Gates (6) is a little more definite and says, 'Excretory organs closed (?), (enteronephric or exonephric) micronephridia in vertically placed clusters on anterior faces of septa 5/6-13/14; from XV posteriorly closed (?), (enteronephric or exonephric) micronephridia on posterior faces of the septa.'

I have examined the nephridial system of this worm both by dissections and serial sections and find that its nephridial system is completely and deeply enteronephric. All the nephridia in the body open into the gut, there being no exonephridia in any part of the body at all.

All the nephridia from segment XIV onwards are septal meronephridia with pre-septal funnels, 20 to 24 per segment, each nephridium having more or less the same size and shape

as that of *Pheretima*; the terminal canals of the nephridia on each side of a septum open into a septal excretory canal, and the two septal canals travel dorsally to open into a continuous excretory duct which is no longer supra-intestinal in position, but has sunk deep into the roof of the intestine to form



TEXT-FIG. 4.

A reconstruction of a few consecutive sections through the intestinal region of *Travescolides chengannures* to show the two septalexcretory canals opening into the intra-typhlosolar excretory duct and the latter opening into the intestinal lumen. *dv*, dorsal vessel; *is*, intersegmental septum; *intw*, intestinal wall; *ited*, intra-typhlosolar excretory duct; *o*, opening of the intra-typhlosolar excretory duct into the lumen of the intestine; *sec*, septal excretory canal; *tnc*, terminal nephridial canals; *ty*, typhlosole. ( $\times$  cir. 118.)

a median intra-typhlosolar excretory duct (Text-fig. 4), opening into the lumen of the intestine at each intersegmental place.

In the pre-clitellar segments, the nine pairs of tufted nephridia in segments V to XIII are also enteronephric in that they open into the lumen of the pharynx and oesophagus by means of nine pairs of ducts. The first pair of tufts lying on each side of the gizzard in segment V gives off a pair of long ductules which travel forward to open into the pharynx in the second

segment, just in front of the nerve-collar. Similarly the next eight pairs of tufted nephridia open by their respective paired ducts into the pharynx and oesophagus.

So far the enteronephric type of excretory system in its best-developed condition in earthworms was known in the genus *Pheretima*, in which it occurs side by side with the exonephric system. But the excretory system of *Travoscolides* is even more specialized than that of *Pheretima* in that it is completely enteronephric, there being no exonephridia at all. Further, it is deeply enteronephric in that the two supra-intestinal excretory ducts have become fused together to form one median duct which is deeply imbedded within the typhlosole.

I have also examined specimens of *Travoscolides duodecimalis* and *Travoscolides pilatus* and find that the nephridial system of these species closely resembles that of *Travoscolides chengannures*, i.e. it is completely and deeply enteronephric.

#### 4. THE ABSENCE OF ENTERONEPHRIC SYSTEM IN *MEGASCOLEX CAERULEUS*.

Bourne (5) described the nephridia of *Megascolex caeruleus* as follows: 'Nephridia are present in the form of minute scattered tubules and may be seen over almost the entire extent of the body-wall. There are no large tufts of tubules.' He further adds that 'the nephridia are most minute, actually smaller than in any *Perichaete* known to me'. Bourne's description is unfortunately not quite correct; he missed the large pharyngeal nephridial tufts in segment IV, just in front of the first septum IV/V. These tufts consist of a large number of funnelless micronephridia, the ductules of which open into the buccal cavity and pharynx, as do the pharyngeal tufts of *Pheretima*; the nephridia throughout the rest of the body are all exonephric integumentary nephridia; in each segment they form two thickly set bands on the inner surface of the body-wall along the anterior and posterior borders of each segment, the middle of the segment being more or less devoid of nephridia. The nephridia are really very minute, each consisting of a short straight lobe and a long twisted loop, like the

integumentary nephridia of *Pheretima*; on an average, the short lobe is  $170\mu$ , while the twisted loop is  $325\mu$  in length, the corresponding lengths in *Pheretima posthuma* being  $100\mu$  and  $182\mu$ , so that they are slightly larger than those of *Pheretima* (*Perichaeta*). But it is interesting that one of the largest and thickest of earthworms should have such minute nephridia, but as they are so numerous, the numbers apparently make up for the minute size of the nephridia.

The enteronephric system is completely absent in this species as in *Megascolex templetonianus* (4). These two species further resemble each other in possessing ventral phagocytic organs. It is for the systematists to decide whether the absence of enteronephric system and the presence of ventral phagocytic organs would still entitle these two species to be retained in the genus *Megascolex*.

#### 5. DISCUSSION AND SUMMARY.

With the discovery of the enteronephric type of excretory system in three more species of *Megascolex* and also in the genus *Travoscolides*, this type of nephridial system is now known to occur in the following earthworms:

a. *Megascolex*—(1) *M. ceylonicus*, (2) *M. sarsinorum*, (3) *M. cochinchensis*, (4) *M. konkannensis*, (5) *M. travancorensis*,<sup>1</sup> (6) *M. trivandranus*,<sup>1</sup> (7) *M. auriculata*,<sup>1</sup> (8) *M. cingulatus*, (9) *M. campester*, and (10) *M. bifoveatus*.

b. *Tonoscolex*—all species.

c. *Lampito mauritii* and *Lampito trilobata*.

d. *Woodwardia bahli*.

e. *Pheretima*—all species.

f. *Travoscolides*—all species.

The best-developed enteronephric system previously known was in *Pheretima*, in which it occurs side by side with the exonephric system. But it has now been found that this type of nephridial system reaches its highest development in *Travo-*

<sup>1</sup> Vidya Vati, "The Enteronephric System in *Megascolex trivandranus*, &c.", 'Proc. National Institute of Sciences, India'. (In the Press.)

scolides in which the nephridia in all parts of the body are exclusively enteronephric, so that *Pheretima* in which this type of nephridial system was first discovered now occupies the second place, the first place being taken by *Travoscolides*. But *Pheretima* still remains unique in that it possesses nephridia attached to both sides of the septa, and the funnels of these nephridia are not attached to the septa but float freely in the coelomic cavity—characters so far found in no other earthworm. The nephridial system of *Travoscolides* affords an example of an organ system which has undergone a complete change of direction, opening exclusively into the gut instead of to the exterior.

It is now possible to visualize the various steps in the evolution of the enteronephric system, although its beginnings are still obscure. The earliest stage is seen in several species of *Megascolex*, like *Megascolex ceylonicus* (4), in which the septal nephridia are few and are confined to the posterior one-third to one-half of the worm; their terminal nephridial canals travel individually along the septa to the mid-dorsal line and open into the intestine either separately, or in groups, or after uniting together to form one canal. The next stage is the formation of a continuous septal excretory canal on each side, into which the septal nephridia open, as in *Megascolex cochinchensis* (3). In both these stages the enteronephric system is still separate from segment to segment, there being no continuity, because there are no continuous supra-intestinal excretory ducts. The next higher stage is represented in *Megascolex campester* and *Megascolex bifoveatus* in which the lateral supra-intestinal excretory ducts appear, one on each side of the dorsal vessel. Of these two species, *Megascolex campester* is more highly evolved than *Megascolex bifoveatus* as the former has septal excretory canals which are absent in the latter. With the appearance of the supra-intestinal ducts the enteronephric nephridial system in the whole worm becomes united and continuous. Although the two supra-intestinal ducts communicate with each other by a transverse ductule in each segment, they remain separate for the greater part of their

length and open into the intestine by two separate vertical canals in each intersegmentum. The next stage is represented in *Pheretima* (2) in which the two lateral supra-intestinal ducts come close together beneath the dorsal vessel, so that they come to occupy a mid-dorsal position and communicate with each other along the greater part of their length; there is only one opening into the intestine in each intersegmentum, but the ancestral condition of two separate openings is still indicated by the fact that the opening is alternately to the right and left of the typhlosole in succeeding segments. In all these earthworms, except in *Megascolex cochinensis*, the septal nephridia (enteronephric) co-exist with the exonephric integumentary nephridia; in *Megascolex cochinensis* the integumentary nephridia are present in the anterior segments but are absent in those segments in which the enteronephridia occur. The highest stage in evolution is reached in *Travoscolides* in which the two supra-intestinal ducts have fused together to form one median duct which has sunk deep into the typhlosole to form the intratyphlosolar excretory duct opening into the intestine by a single median opening on the typhlosole in each segment; further, there is a complete disappearance of the integumentary nephridia not only from the post-clitellar but also from the pre-clitellar segments, the nine pairs of tufted nephridia on either side of the gizzard and oesophagus opening into the pharynx and oesophagus.

The nephridial system of *Travoscolides*, therefore, shows extreme specialization. It is difficult to say which is more efficient all round—the nephridial system of *Pheretima* or that of *Travoscolides*. If conservation of water is the function of the enteronephric system, certainly the nephridial system of *Travoscolides* is more efficient; but it would seem that *Pheretima* has, so to speak, two strings to its bow, an exonephric and enteronephric one, while *Travoscolides* has only one, an enteronephric one. In the number of species and in distribution, *Pheretima* has been far more successful than *Travoscolides* which is confined only to a very small area in the extreme south of India—Cochin and Travancore.

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# Development of Olfactory Organ in *Rana nigromaculata*.

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With 27 Text-figs.

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## INTRODUCTION.

SINCE Goette's classical paper on the development of the toad (Goette, 1875) there have appeared many accounts of the ontogeny of the olfactory organ of Amphibia. The most thorough-going work has been that of Hinsberg (1901), who followed very closely the early developmental steps but not the later ones. In the developmental history of the amphibian nasal organ two points in particular have arrested the attention of students of comparative embryology and caused a good deal of controversy. One is the existence of the oro-nasal groove, the other is the location of the choanal opening in the oral cavity. Kurepina (1931) claimed that there is present in the amphibian larva an oro-nasal groove, comparable to the nasal groove in embryos of the Amniota, while Hinsberg (1901), Kawagae (1933), and Schneider (1935) were unable to find such a groove. Kurepina also found that the choana opens into the ectodermal part of the oral cavity as it does in all Amniota. In this he was supported by Watanabe (1936). On the other hand, Goette (1875), Hinsberg (1901), and others (Fahrenheit, 1925; Kawagae, 1933; Schneider, 1935) contended that the



choanal opening is in the endoderm. However, neither side has given conclusive evidence.

*Rana nigromaculata* Hallowell is a common frog in North China. Its olfactory organ has not been investigated. The aim of the present paper is to make a thorough and complete study of the development of that organ and to see if fresh light can be thrown on those controversial points mentioned above.

The present investigation was conducted in the Biology Department, the National University of Peking, Peiping. When the work was nearly completed, it was interrupted by the Japanese invasion and was finally completed in Kunming.

#### MATERIAL AND TECHNIQUE.

*Rana nigromaculata* is very abundant in Peiping. One can get its eggs and tadpoles in ponds, pools, and rice fields from April to July. A complete series of stages, ranging from larvae 2 mm. long to young frogs was collected. Identification was based on descriptions in Boring, Liu, and Chou (1932, pp. 39-49). The body-length was measured and recorded. One-half of the larvae of the same stage was preserved in 10 per cent. formalin for gross observation and comparison with sections. The other half was fixed in Spuler's fluid (Müller's solution, 70 c.c., saturated sublimate solution, 30 c.c., concentrated formalin, 10 c.c., and glacial acetic acid, 2 c.c.) or in a mixture of acetic acid and sublimate (saturated sublimate solution, 35 c.c. and glacial acetic acid, 20 c.c.). Each fixative was prepared immediately before use. The fixed material was imbedded in paraffin. Both cross and sagittal serial sections were cut 5-9 $\mu$  in thickness and stained with Delafield's haematoxylin or a combination of Delafield's haematoxylin and eosin.

It was necessary to employ reconstructions. For this purpose I tried to use the blotting-paper method described in Gage (1936, pp. 511-20) instead of the wax-plate method. But I had trouble in cutting. I then tried to infiltrate a piece of blotting paper with beeswax which made the paper much firmer. The cutting was very easily done with the knife used by Chinese wood-carvers. The method is as follows:

A sheet of blotting paper ( $48 \times 61$  cm.) was cut into three strips and immersed in a bath of melted beeswax, to 100 parts of which 5 parts of paraffin were added. After two minutes they were taken out of the bath and both sides of the paper were quickly wiped with a piece of cloth in order to make the waxed paper even and smooth. Every strip of the waxed paper was cut again into five. To determine the thickness of the waxed paper, every piece was measured in 8 different places with a micrometer caliper. The mean of the 120 readings ( $15 \times 8$ ) was taken to be the correct thickness. The most suitable thickness for cutting a complicated model was 0.4 to 0.5 mm. Thinner pieces would be, of course, easier to cut, but involved more work. Paper thicker than 0.5 mm. was employed only for making simple and large models. For sections cut  $8\mu$  thick, paper 0.4 mm. in thickness was just right for magnifying them  $50\times$ ; two thicknesses of paper for  $100\times$ .

Serial drawings were made on the waxed blotting paper with a fine needle under an Edinger drawing machine or a 'Panphot' (manufactured by Leitz). It was found easier, however, to make sketches on a piece of blotting paper with a sharp pencil before infiltration with wax. Further treatment is just the same as in the wax-plate method.

Models constructed with wax-infiltrated paper are very strong. This turned out to be very fortunate. Since the Sino-Japanese war, the models together with other materials had to be hurriedly shipped South. On the way they were subjected to rough handling and subtropical heat. If the models had been made of wax, they would have been hopelessly ruined. My models arrived, however, in perfectly good condition.

## DEVELOPMENTAL ANATOMY.

### A. Olfactory Cavities.

The course of development of the olfactory cavities may be conveniently divided into four stages.

#### 1. First Stage.

From the Inception of the Olfactory Anlage to

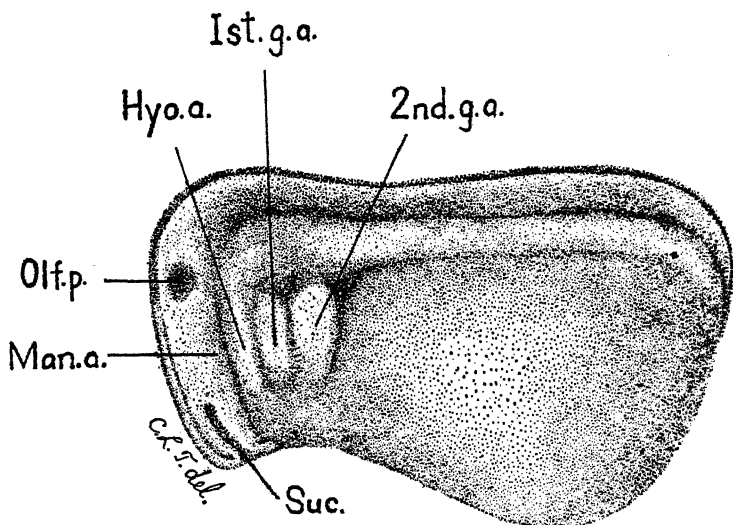
the Formation of the Olfactory Placode (length of larva, 2.5–5 mm.).—When the neural groove just closes to form a tube, the ectoderm of the head of the larva shows clearly the surface and the sensory layer (Goette, 1875; Corning, 1899). The larva at this stage is about 2.5 mm. long. The anlage of the olfactory placode arises as a pair of thickenings from the sensory layer of the ectoderm at the top of the head and in front of the optic vesicle. When strongly magnified, the external surface of this anlage can be barely made out as a slight rounded depression (Text-fig. 6). A larva of about 2.8 mm. in length shows the primitive placode definitely depressed to form the olfactory pit, which can be already seen under  $24\times$  magnification (Text-fig. 1). Hinsberg (1901) working on *Rana fusca* stated that the olfactory pit appears only when the larva reaches 5 mm. in length. In *nigromaculata* it appears then much earlier. No further changes could be observed at this stage except the increase in dimension of the placode, and that with the enlargement of the head at the brain region the placode which first appeared dorsally is displaced ventrally. Hinsberg (1901) discussed at length the question, whether the surface layer of ectoderm takes part in the formation of olfactory placode and concludes that in *fusca* the placode is derived solely from the sensory layer. My findings in *Rana nigromaculata* completely support those of Hinsberg.

In the head of the larva of *Rana nigromaculata* there is also a forehead streak, the 'Stirnstreifen' of Hinsberg (1901). It will be dealt with in a separate paper, for it has nothing to do with the development of the olfactory organ.

Fresh specimens, serial sections and models of the head in the first and later stages were examined, but no trace of the oronasal groove of Kurepina (1931) was found.

## 2. Second Stage.

(a) Formation of the Nasal Lumina (length of larva, 5–9.5 mm.).—The olfactory pit remains a shallow depression as long as the lens is still attached to the sensory layer of the ectoderm. The formation of the nasal lumina synchronizes with the separation of the lens from the sensory



TEXT-FIG. 1.

Lateral view of the larva (2.8 mm. long).  $\times 30$ . *1stga*, first gill arch; *Hyoa*, hyoid arch; *Mana*, mandibular arch; *Olfp*, olfactory pit; *2ndga*, second gill arch; *Suc*, sucker.

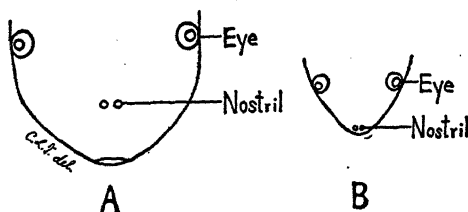
#### ABBREVIATIONS.

(Text-figs. 6-12 drawn with the aid of a camera lucida and magnified  $86\times$ .)

*Antlos*, anterior lower sac; *Cavinf*, cavum inferius; *Cavmed*, cavum medium; *Cavprinc*, cavum principale; *Cho*, choana; *Cle*, clefts; *Cons*, constriction; *Dien*, diencephalon; *Dorl*, dorsal lumen; *Ect*, ectoderm; *Ent*, endoderm; *Entrean*, entrance canal; *Epi*, epiphysis; *Ethch*, ethmoidal part of chondrocranium; *Extnar*, external naris; *Hypo*, hypophysis; *Inf*, infundibulum of nasal cavity; *Inf'*, infundibulum of brain; *Latap*, lateral appendix; *Latgl*, lateral nasal gland; *Latgr*, lateral groove; *Latp*, lateral portion; *Llid*, lower eyelid; *Llip*, lower lip; *Medgl*, medial nasal gland; *Medp*, medial portion; *Mes*, mesencephalon; *Met*, metencephalon; *Midl*, middle lumen; *Nar*, nasal rim; *Nasduct*, naso-lacrymal duct; *Nasduct'*, external opening of naso-lacrymal duct; *Noto*, notochord; *Olfp*, olfactory pit; *Olfpl*, olfactory placode; *Optves*, optic vesicle; *Orca*, oral cavity; *Orep*, oral epithelium; *Palep*, palate epithelium; *Phagl*, pharyngeal gland; *Pham*, pharyngeal membrane; *Phar*, pharynx; *Postlos*, posterior lower sac; *Postpro*, posterior prolongation; *Pricho*, primitive choana; *Proj*, projection; *Pros*, prosencephalon; *Recalar*, recessus alaris; *Reclat*, recessus lateralis; *Recmmed*, recessus medialis; *Recsac*, recessus sacciformis; *Senlay*, sensory layer; *Sk*, skin; *Surlay*, surface layer; *Suplatgr*, super-lateral groove; *Ulip*, upper lip; *Ups*, upper sac; *Venl*, ventral lumen.

layer. The nasal pit extends inward and upward to form a tube, the enlarged end of which forms the dorsal lumen (Text-fig. 7).

After the formation of the dorsal lumen, the neighbouring cells behind it are immediately differentiated to form a blind sac, which is called the lateral appendix. At the time of its formation it lies just at the dorso-lateral region of the placode (Text-fig. 8). Later, with the more rapid expansion of the



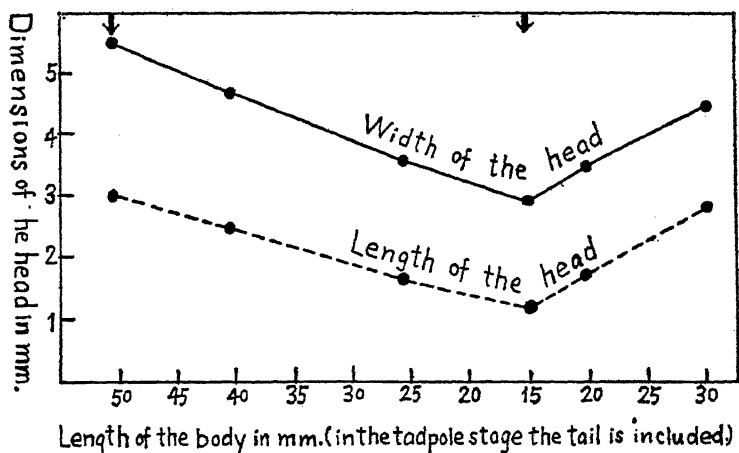
TEXT-FIG. 2.

Comparative dimensions of the head before (A) and after (B) the metamorphosis.  $\times 3$ .

placode to the dorsal and medial side, the lateral appendix is gradually shifted latero-posteriorly and a marked constriction appears between it and the placode (Text-figs. 10 and 18). The part of the placode posterior to the lateral appendix is again differentiated into two portions, a thin lateral one and a thick medial one. Between them there originate several minute clefts (Text-fig. 8). These are at first discontinuous but later communicate with one another to form the ventral lumen posterior to the olfactory pit (Text-fig. 10). Contemporaneous with the appearance of the clefts, the olfactory pit begins to extend inward and gives rise to another lumen, the middle lumen, which is immediately anterior to the dorsal lumen (Text-fig. 9) and runs ventro-posteriorly. Its medial wall is derived from the olfactory placode, while its lateral wall is from inward extension of the skin (Text-fig. 10). It communicates with the dorsal lumen from the very beginning (the larva at this time is 7 mm. in length) and with the ventral one when the clefts run together to form a whole lumen (the length of the larva now is 8.5 mm.). Between the middle and ventral lumen there is a small inward

projection, which is particularly plain in nigromaculata (Text-figs. 10 and 14). This projection serves at this time as a boundary between the middle and ventral lumen. Its subsequent development will be described later.

(b) Formation of the Primitive Choana (length of larva 5.5-9.5 mm.).—The formation of the primitive choana in the oral cavity is closely related to the formation and

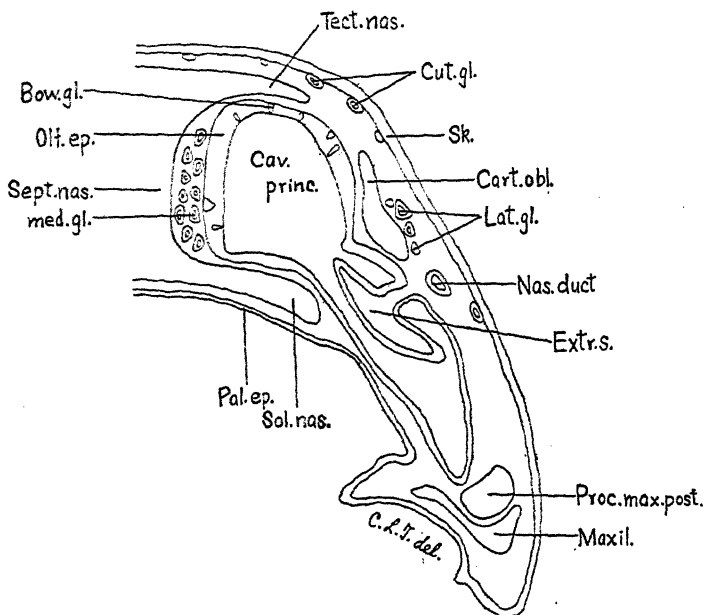


TEXT-FIG. 3.

Graphic representation of the changes in the dimensions of the head in relation to metamorphosis. The arrows mark the beginning and end of metamorphosis.

rupture of the pharyngeal membrane. The latter is formed by fusion of the intruded ectoderm and the protruded endoderm. As the brain expands, the forehead is thrust forward and downward, so that the pharyngeal membrane is then shifted backward (Text-fig. 11). At each side of the pharyngeal membrane the two embryonic layers meet to form the lateral walls of the primitive oral cavity. The method of fusion of these two layers is somewhat peculiar. The endoderm protrudes above, while the ectoderm intrudes below (Text-fig. 13). As the result of this kind of fusion, the meeting line of the two layers is not vertical but runs obliquely from the dorso-anterior towards the

ventro-posterior (Text-figs. 11 and 14). Therefore, in the roof of the oral cavity the endoderm extends much more anteriorly than it does in the floor (Text-figs. 12 and 14). By the difference in microscopic structure one can easily distinguish the two layers. The ectoderm in the oral cavity is thicker and in a given



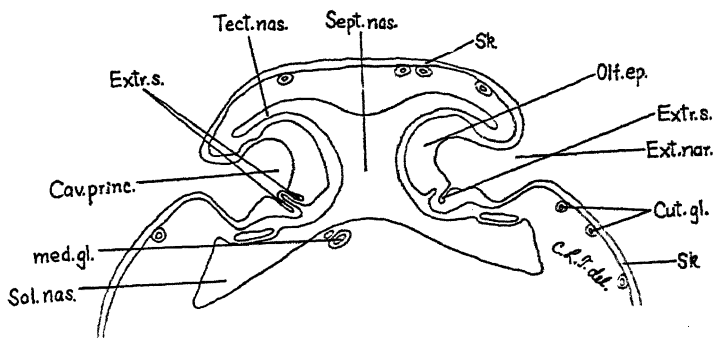
TEXT-FIG. 4.

Camera lucida outline of cross-section of the head through the middle region of nasal cavity showing an extra blind sac.  $\times 38$ . *Bowl*, Bowman's gland; *Cartobl*, cartilago obliqua; *Cutgl*, cutaneous gland; *Extrs*, extra blind sac; *Maxil*, maxilla; *Olfep*, olfactory epithelium; *Promaxpost*, processus maxillaris posterior; *Septnas*, septum nasi; *Solnas*, solum nasi; *Tectnas*, tectum nasi. For other abbreviations, see list of abbreviations.

area contains many more cells, the nuclei of which are crowded together. The endoderm is a thin layer; its cells are not so crowded and contain many yolk-granules. This layer takes the stain less deeply (Text-fig. 12).

The olfactory placode in the 5.5 mm. larva shows a small

prolongation at its most posterior end. This prolongation extends ventro-posteriorly towards the oral cavity (Text-fig. 13). The tip of it is later thrust into the endodermal part of the oral cavity at the point where the lateral wall meets the roof (Text-figs. 12 and 14). This occurs just after the rupture of the pharyngeal membrane. At this stage, the ventral nasal lumen



TEXT-FIG. 5.

Camera lucida outline of cross-section of another head at the level of the external naris showing three extra blind sacs.  $\times 35$ . Labels like those used in Text-fig. 4.

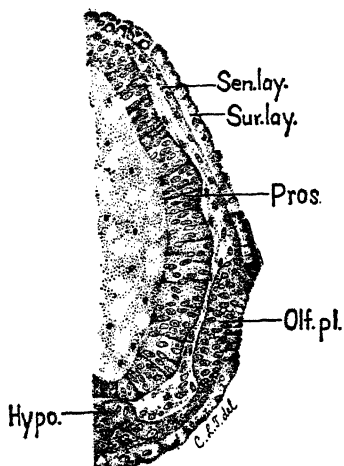
is still represented by the discontinuous clefts (p. 66). After these clefts have run together to form the ventral lumen, it extends into the prolongation and opens at its distal end into the oral cavity. This distal opening is the primitive choana. Since the tip of the prolongation grows into the portion of the oral cavity lined by endoderm, the choana opens definitely into the endoderm.

The choana possesses medial extension on the palate in the form of a transverse groove (Text-fig. 16). The roof of this groove is derived from the posterior prolongation of the olfactory placode, while the walls flanking it consist of the mixture of the placodal and endodermal tissues.

### 3. Third Stage.

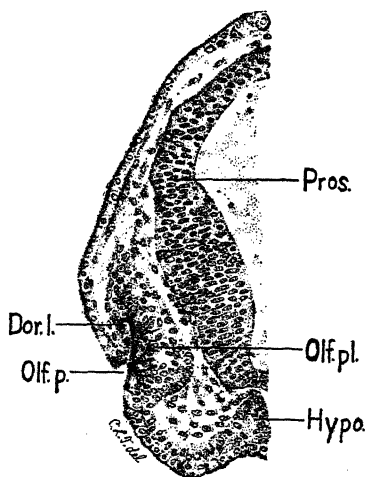
Formation of Blind Sacs and Changes of the External Naris and the Primitive Choana (length





TEXT-FIG. 6.

Cross-section of a portion of the head through the olfactory placode (length of larva, 2.5 mm.).

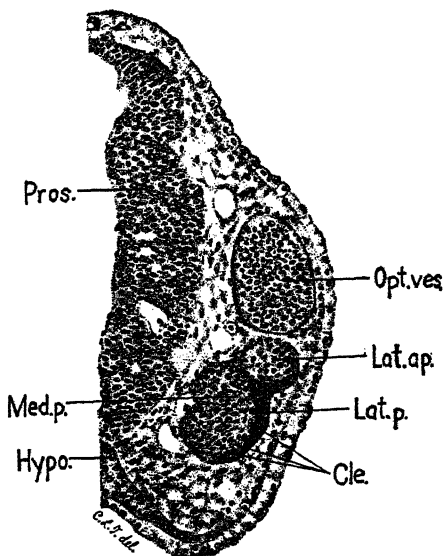


TEXT-FIG. 7.

The same, through the olfactory pit and dorsal lumen of the olfactory placode (length of larva, 7 mm.).

of larva, 9.5-50 mm.).—Up to the end of the second stage the olfactory placode with its lumina is more or less straight (Text-fig. 17). This simple structure undergoes profound changes to become a very complicated organ.

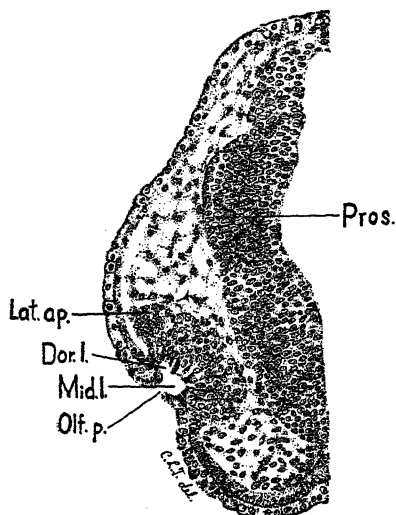
The embryonic olfactory organ as it appears at the end of the third stage may be divided according to natural demarcations



TEXT-FIG. 8.

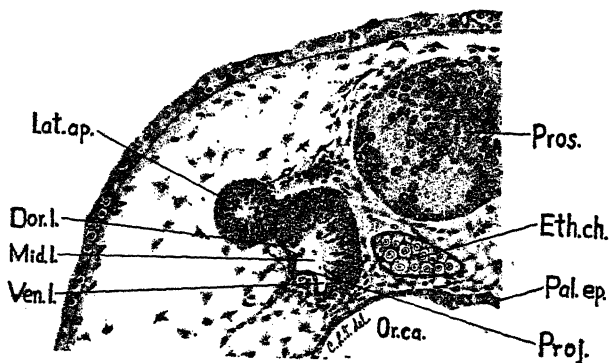
The same, through lateral appendix of the olfactory placode  
(length of larva, 8.5 mm.).

into three blind sacs. Using the designations of Hinsberg (1901) the three sacs are called the upper sac, the anterior lower sac, and the posterior lower sac (Text-figs. 21 and 22). The upper sac includes the external naris and contains the middle nasal lumen. At first, it is the largest sac (Text-fig. 17). The anterior lower sac is a small one situated ventro-anteriorly to the upper sac. The posterior lower sac is under the upper sac. It contains the ventral lumen and includes the choana. The three sacs arise in the following manner.



TEXT-FIG. 9.

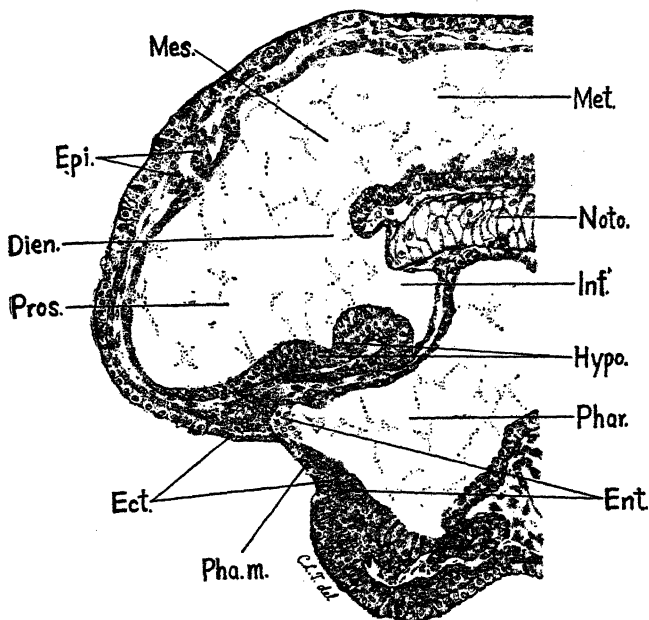
The same, through the communication of dorsal and middle lumen of the olfactory placode (length of larva, 8.5 mm.).



TEXT-FIG. 10.

The same, through the communication of dorsal, middle, and central lumen of the olfactory placode (length of larva, 9.5 mm.).

The part of the olfactory placode around the middle nasal lumen enlarges and the lumen expands along with it, thus bringing about the formation of the upper sac. Contemporaneous with the coming into being of the upper sac, the placodal region around the ventral lumen together with the



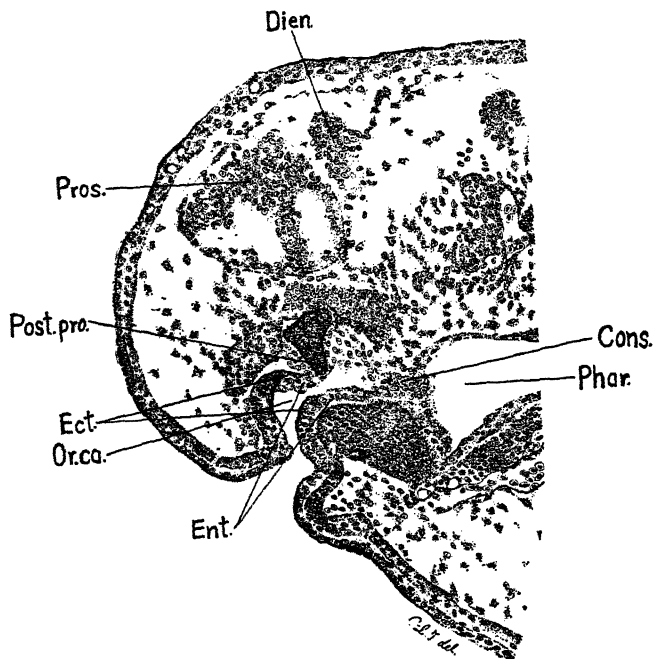
TEXT-FIG. 11.

Sagittal section through the pharyngeal membrane (length of larva, 5.5 mm.).

posterior prolongation and the primitive choana (into which the ventral lumen opens) enlarges and the whole complex now becomes the posterior lower sac. At the beginning it is not marked off from the upper sac (Text-fig. 17).

It will be recalled that between the middle and ventral lumen there is an inward projection (p. 66). As the upper and posterior lower sacs are being formed, this inward projection also enlarges. When viewed from the outside of the olfactory organ

at this stage, this inward projection is a small depression (Text-fig. 17). Following the expansion of the upper and the posterior lower sacs this depression increases both in length and in depth. This structure I designate as the lateral groove. It marks off



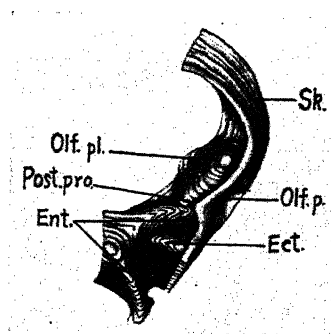
TEXT-FIG. 12.

The same, through the posterior prolongation of the olfactory placode. (The posterior prolongation is thrust into the entodermal part of the oral cavity. The entodermal tissue behind the prolongation gives a tangential view of the upper corner of the oral cavity, and thus appears as if it were a thick transection. Length of larva, 7.5 mm.)

the upper sac on the outer side from the posterior lower sac (Text-fig. 18). In the medial region the upper sac projects out and hangs over the latter, though on that side there is no definite groove to mark them off (Text-fig. 18).

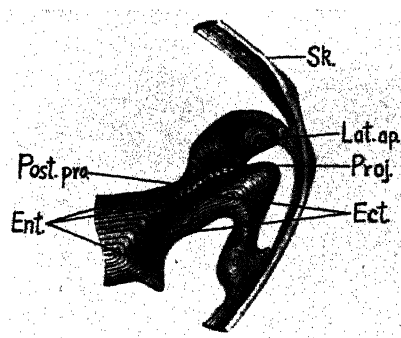
The anterior lower sac is simply an evaginated part of the ventro-anterior region of the upper sac (Text-fig. 17). It pro-

trudes forward under the upper sac. In the early stage, this sac looks somewhat like a flattened egg.



TEXT-FIG. 13.

Lateral view of a model (made from the same specimen a section of which is illustrated in Text-fig. 11) showing the olfactory organ and the lateral wall of the primitive oral cavity.  $\times 35$ .



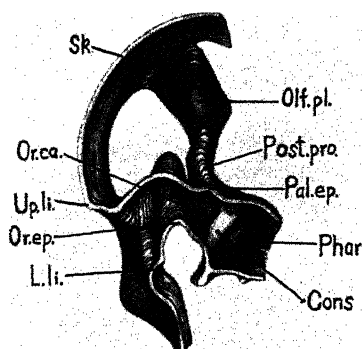
TEXT-FIG. 14.

Lateral view of a model showing the olfactory organ and the lateral wall of the oral cavity. The oblique meeting-line of the ectoderm and entoderm is very clear (length of larva, 8.5 mm.).  $\times 35$ .

After the formation of the three blind sacs, the olfactory organ undergoes the following changes.

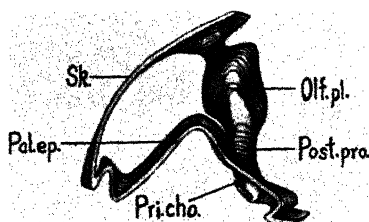
The head as a whole increases more rapidly in size than the

olfactory organ. As a consequence there is now intercalated between the external naris and the middle lumen (which were previously in close contact) a canal formed by the invaginated



TEXT-FIG. 15.

Medial view of a model (made from the same specimen a section of which is illustrated in Text-fig. 12) showing the olfactory organ, oral cavity, and the entodermal constriction of the latter.  $\times 35$ .



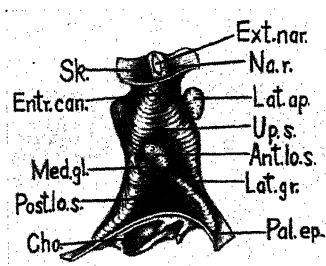
TEXT-FIG. 16.

Antero-medial view of a model showing the olfactory organ and the primitive choana in the roof of the oral cavity (length of larva, 9.5 mm.).  $\times 35$ .

skin inside the naris. This canal is the entrance canal (Text-fig. 17). The surrounding skin of the external naris is raised to form a rim around it (Text-fig. 17). This rim exists up to the time when the larva is 18 mm. long; then it begins to disappear.

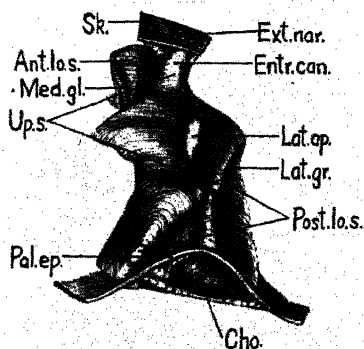
The larva of 25 mm. in length shows no trace of such a rim around the nasal opening.

After the disappearance of the rim the entrance canal dilates (Text-fig. 18). The upper part of it extends dorso-laterally and



TEXT-FIG. 17.

Anterior view of a model showing the beginning of the differentiation of the olfactory organ into three embryonic blind sacs (length of larva, 11 mm.).  $\times 25$ .

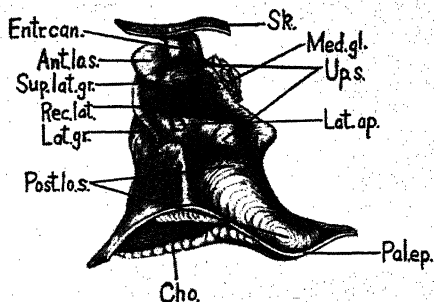


TEXT-FIG. 18.

Dorso-lateral view of a model showing the formation of the lateral groove (length of larva, 22 mm.).  $\times 25$ .

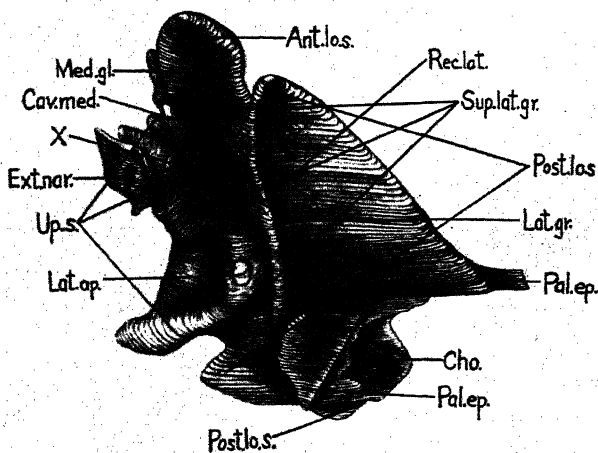
constitutes a small sac (Text-fig. 20, *x*) which in the next developmental stage gives rise to several small structures (see p. 83). The lower part extends in the opposite direction—to the ventro-medial—to form another blind sac which is thrust





TEXT-FIG. 19.

Latero-posterior view of a model showing the formation of the super-lateral groove and recessus lateralis. At this stage the upper sac is clearly demarcated from the posterior lower sac (length of larva, 41 mm.).  $\times 25$ .



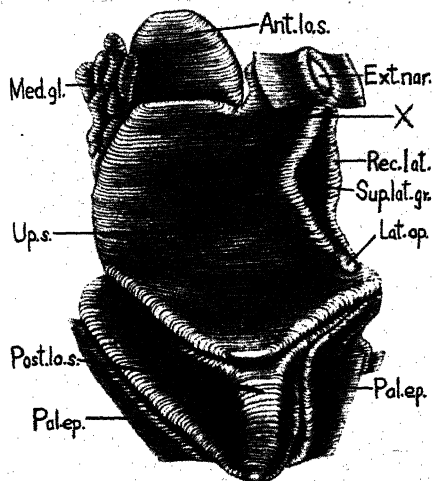
TEXT-FIG. 20.

Lateral view of a model made from the olfactory organ of a specimen just before metamorphosis (length of larva, 50 mm.).  $\times 25$ .

between the upper and anterior lower sac and becomes the cavum medium in adult (Text-fig. 20). Concomitant with the

above changes the external naris is shifting sideways so that the two nasal openings which were close together become at the end of this stage wide apart (Text-fig. 21).

The lateral appendix, as described above (p. 66), is the earliest developed blind sac. It increases very rapidly at the beginning and reaches the highest development at the end of



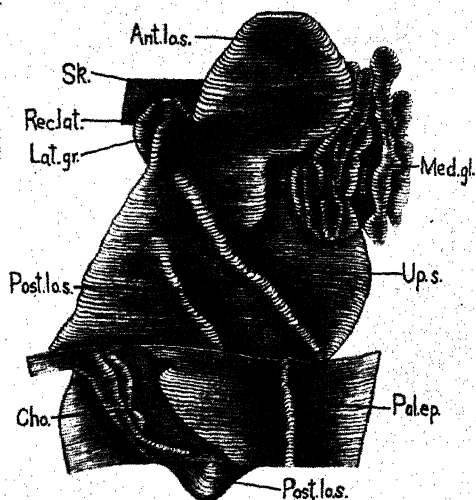
TEXT-FIG. 21.

Dorsal view of the same model.

the second stage. It then remains stationary. Upon the development of the upper sac it is gradually shifted to the lateral and posterior (Text-fig. 18). At this time it starts also to degenerate, becoming increasingly smaller (Text-figs. 19 and 20) until it is obliterated by the end of the metamorphosis. It is remarkable that the lateral appendix, which differentiates very early and, for a time, is the only blind sac in the early larval olfactory organ, should completely disappear in the course of further development. Watanabe (1936) is of the opinion that it may be a sense organ for the larval life, but he gives no evidence to support his conjecture.

At the beginning of the disappearance of the lateral appendix the posterior part of the upper sac which is heretofore continuous with the posterior lower sac (Text-fig. 18) becomes elevated and marked off from the lower one (Text-figs. 19, 20, and 21).

Just above the lateral groove and under the lateral appendix there arises now another groove which extends anteriorly up



TEXT-FIG. 22.

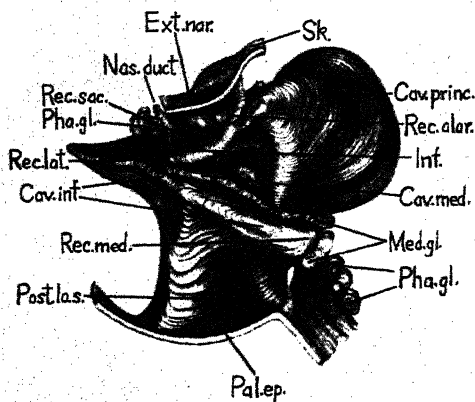
Ventral view of the same model.

to the proximal end of the anterior lower sac (Text-figs. 19 and 20). I call it the super-lateral groove, as it is above that structure. The part of the lateral wall of the upper sac between these two grooves is now separated from the rest of the wall and becomes a ledge overhanging the lateral wall of the posterior lower sac. It becomes the recessus lateralis in the adult (Text-figs. 19 and 20).

The anterior lower sac now increases and extends more anteriorly than in other directions, while the upper sac extends in all directions but very little anteriorly. The result is that the former sac lies far to the front at the end of this stage

(Text-figs. 20, 21, and 22). In the larval stage its communication is always with the middle lumen of the upper sac. In the adult it becomes connected with the recessus lateralis, which is a derivative of the upper sac. These changes will be described in the fourth stage.

As pointed out in a preceding paragraph, the posterior lower



TEXT-FIG. 23.

Anterior view of a model made from the olfactory organ of a specimen during metamorphosis (length of larva, 38 mm.—tail has been resorbed).  $\times 25$ .

sac is not fully separated from the upper sac until the end of the present stage. It expands rapidly, so that at the end of this stage it outstrips the upper sac in size and becomes the largest sac of the time. The expansive growth is far greater at the posterior part, and its form at the end of this stage is roughly a triangular pyramid with its apex directed anteriorly (Text-fig. 22).

With the expansion of the posterior lower sac, the primitive choana also enlarges until the narrow groove becomes quite a large irregular opening (Text-fig. 22). The projecting side walls of the former groove persist for a time, but, towards the beginning of the fourth stage, completely disappear so that the edge of the choana is now perfectly smooth (Text-fig. 25).

#### 4. Fourth Stage.

From the Beginning of the Metamorphosis to the Adult (from larva of 50 mm. in length to adult frog).—When the tadpole reaches 50 mm. in length, its tail begins to be resorbed. There occurs a great change in the nasal cavity. It now undergoes simplification in outline and, at first, noticeable shrinkage in size. This decrease in size is understandable when we compare the head of the tadpole just before metamorphosis with the young frog. The head of the tadpole is much larger and has a rounded outline about the mouth (Text-fig. 2 A). After metamorphosis the head has shrunk in all dimensions and has a triangular outline (Text-fig. 2 B). The change in the size of the head is graphically represented in the Text-fig. 3.

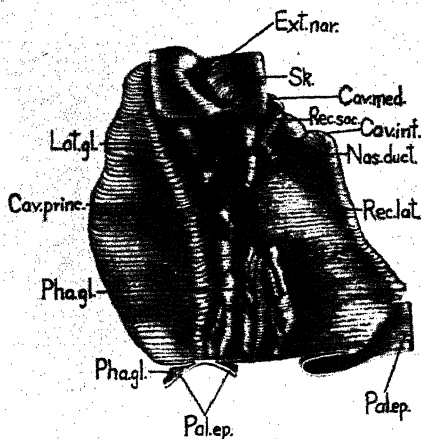
The shrinkage of the head is evidently related to the emergence of the animal from water, which is probably accompanied by dehydration. The connective tissue in the head was very loose in the larva, but becomes compact in the young frog. The simultaneous decrease in the size of the olfactory organ may also be due to the loss of water from its tissues. This point cannot be established histologically, however, since measurements of the cells of the organ before and after metamorphosis do not reveal any shrinkage in their size.

The entire nasal cavity appears also to be shifted forward after metamorphosis and the nostrils are now at the very tip of the head (Text-fig. 2 A and B). This is brought about by the fact that the upper lip now completely folds under as the mouth widens.

The shape of the olfactory organ at the end of the last stage is very irregular and the three sacs are distinct from one another (Text-figs. 21 and 22). During metamorphosis the furrows separating the upper and posterior lower sacs along the medial and posterior region (Text-fig. 21) become smoothed out as a result of distension of the sacs. The elevated posterior part of the upper sac and the lateral appendix lose their identity and the whole of the upper sac and the main portion of the posterior lower sac merge into one large cavity, designated as the chief cavity or *cavum principale* in the mature organ (Text-

figs. 24 and 25). It is flattened dorso-ventrally, shifts medially, and also extends far anteriorly over the anterior lower sac (Text-fig. 25).

The super-lateral groove separating the former upper sac and the posterior lower sac at their lateral parts remains, how-



TEXT-FIG. 24.

Dorsal view of the same model.

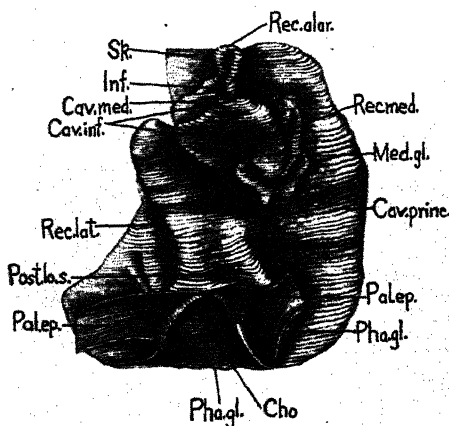
ever. The whole lateral wall of the posterior lower sac extends lateralwards carrying the ledge along with it, forms a side extension of the cavum principale, and is designated as recessus lateralis (Text-figs. 24 and 26).

The anterior lower sac lies now completely under the cavum principale. The main part of it together with the foremost extension of the recessus lateralis, which projects until it is level with the anterior lower sac, constitutes the cavum inferius of the mature organ (Text-figs. 23 and 25), while the medio-posterior portion of the sac is called recessus medialis (Text-figs. 25 and 27).

The small sac derived from the upper part of the entrance canal, and situated under the external naris (Text-fig. 20, *x*) expands to form the anlagen of the recessus alaris, infundibulum

and recessus sacciformis (Text-fig. 23). The cavum medium (p. 78) is then shifted to the lateral under the infundibulum (Text-fig. 23) instead of its original position. The external naris enlarges and along with the cavum principale extends forward (Text-figs. 24 and 26).

The olfactory organ increases in size along with the growth



TEXT-FIG. 25.

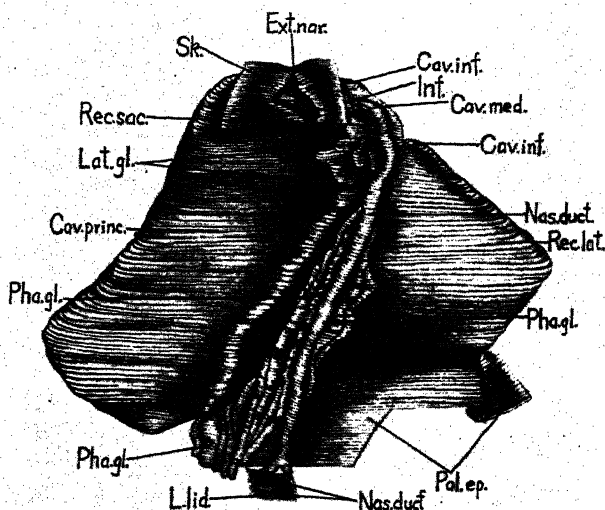
Ventral view of the same model.

of the head. In a later stage it is still further flattened out dorso-ventrally. The cavum principale at its hind portion stretches medially and the recessus lateralis extends further laterally. The choana becomes greatly appressed (Text-fig. 27). It is now relatively large, but in the mature organ its relative size to that of the nasal cavity is much smaller (Tsui, 1935, fig. 6).

From this point on, the structure of the nasal cavity undergoes no important change. In the adult stage, the olfactory organ of *Rana nigromaculata* is essentially the same as that of *fusca* and *esculenta* (Tsui, 1935). It is, therefore, unnecessary to carry the description farther.

**Extra Blind Sacs.**—Extra blind sacs have been occasionally found in the nasal cavity. In three cases such sacs were

observed—all in the stage when the cartilaginous nasal capsule is already formed. In one specimen an invaginated sac is present in the cavum principale (Text-fig. 4). In the other two, one or two small folds occur in the medio-anterior part of that cavity (Text-fig. 5). All these extra pouches are symmetrically present in both nasal cavities. The formation of these unusual structures is perhaps due to the fact that the cells in certain



TEXT-FIG. 26.

Dorsal view of a model made from the olfactory organ of a young frog (length of the body, 20 mm.).  $\times 25$ .

regions of the olfactory epithelium increase abnormally. On account of the presence of the cartilaginous nasal capsule, the epithelium cannot extend freely and, as a result, a blind sac is formed. An undetermined blind sac was reported in *fusca* (Tsui, 1935). It was probably formed in the same way.

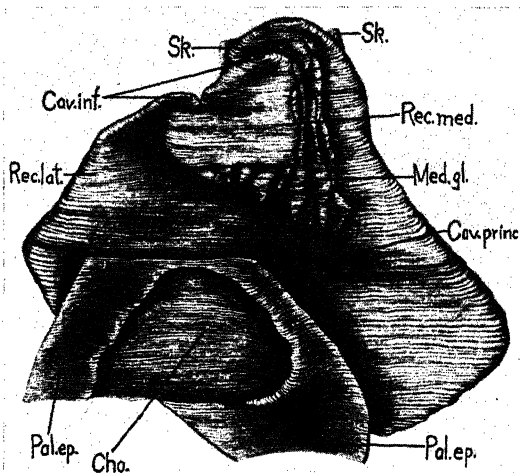
## B. Accessory Glands and Naso-lacrymal Ducts.

1. Naso-lacrymal Ducts.—The development of the naso-lacrymal duct is very sudden. It arises in the dorso-lateral



part of the cavum principale behind the external naris (Text-fig. 24). Due to the formation of the recessus sacciformis it is pushed farther laterally and is connected with the latero-posterior end of the cavum medium (Text-fig. 26). It lengthens posteriorly, the distal end of the duct bifurcates, and the two branches open into the lower eyelid (Text-fig. 26).

According to Born (1876), this duct in the amphibians he



TEXT-FIG. 27.

Ventral view of the same model.

examined is first solid and hollows out to form a duct. Transverse serial sections were made of the duct of the present species. It was found that the proximal end of this duct is at first solid. The duct lengthens very rapidly and when it reaches the eyelid, it is already hollow along most of its length. Finally the proximal end also becomes hollow. The formation of the lumen seems to begin from the distal end.

2. Lateral Nasal Gland.—At the same level as, and medially to, the naso-lacrymal duct there arises the lateral nasal gland. It is a small diverticulum (Text-figs. 24 and 26). Soon it increases in size, and branches laterally towards the naso-

lacrymal duct to become a compound tubulo-acinous gland. When mature, it occupies a great surface between the cavum principale and recessus lateralis and covers the anterior part of the naso-lacrymal duct (Tsui, 1935, fig. 7).

3. Medial Nasal Gland.—At the beginning of the third developmental stage of the olfactory organ, the cells of the olfactory epithelium in the medio-posterior angle of the anterior lower sac differentiate gradually into a tiny diverticulum composed of simple epithelium. This is the medial nasal gland (Text-fig. 17). It grows forward and chiefly along the medial edge of the anterior lower sac until it is on a level with the distal end of the latter (Text-fig. 18). The structure is quite simple, containing only a few branched tubules. The proximal tubule becomes the short duct of this gland, which opens at the place of its origin. The distal tubules then branch farther and extend now chiefly medially and posteriorly, but very little dorsally, ventrally, or anteriorly. As the anterior lower sac later extends more anteriorly and medially, the gland assumes relatively a more posterior position (Text-fig. 22). Later it ramifies profusely to become a small branched tubular gland. It now grows mainly backwards and laterally. Still later the cartilaginous nasal capsule is formed and closely encases the olfactory organ including this gland, restricting its growth along the medial and posterior region. Consequently the further growth of the gland is chiefly towards the front (Text-figs. 25 and 27).

It may be pointed out here that during metamorphosis when the cavity shrinks in size (p. 82), this gland also undergoes considerable shrinkage. A detailed cytological investigation of this gland and the lateral nasal gland will be presented in a separate paper.

4. Glands of Bowman.—Glands of Bowman are imbedded in the epithelium of the cavum principale. They do not appear until the larva is 46 mm. long. The mouth part of the gland arises earlier than the body. Moreover, the glands do not arise simultaneously in the whole cavum principale, but appear in the posterior part of it earlier than in the anterior.

5. Pharyngeal Gland.—The pharyngeal gland appears

just at the beginning of the fourth stage behind the lateral nasal gland along the groove separating *cavum principale* and *recessus lateralis* (called the super-lateral groove). It extends posteriorly and runs over the roof of the choana and down the medial wall of it (Text-figs. 24 and 26).

#### DISCUSSION.

Careful as Hinsberg's work (1901) was, he left a big gap in the ontogeny of the nasal organ. The changes described in the fourth stage were completely overlooked by that author. The shrinkage of the organ during metamorphosis is interesting and, as far as I know, has not been reported by previous writers.

With regard to the question whether the primitive choana opens into the endodermal or ectodermal part of the oral cavity, it is sufficient to discuss the investigations of two authors, Kurepina (1931) and Watanabe (1936), who paid special attention to this point.

Kurepina made reconstructions from sagittal sections. Such sections show plainly a lateral constriction on either side of the oral cavity. Kurepina took this constriction to be the vestige of the pharyngeal membrane. Since this membrane marks the meeting place of the two embryonic layers, Kurepina concludes that the choanal opening, which is anterior to the constriction, must be ectodermal. In *Rana nigromaculata* such a pair of lateral constrictions is also present (Text-fig. 15). My reconstructed model of the head of *nigromaculata* at this stage shows structures identical with that illustrated by Kurepina (cf. Text-fig. 15 with Kurepina's fig. 15, p. 26). But the lateral constriction in *nigromaculata* does not represent a vestige of the pharyngeal membrane. It is a constriction near the front end of the primitive pharynx. The endoderm on the upper part of the oral cavity stretches far beyond it and the pharyngeal membrane is formed in front of this pair of lateral constrictions (Text-figs. 12, 13, and 14). At the time of the formation of the primitive choana this membrane has ruptured and left no vestige to serve as a guide. Microscopic observations show, however, that the tissue into which the choana opens is definitely endodermal. Evidently Kurepina made no careful microscopic

observation of his sections, but relied solely on his models which could not reveal detailed histological structure. One has reason to suspect that what is true of *Rana nigromaculata* also obtains in *Pelobates fuscus*, which he figured, and perhaps in other amphibians he studied.

Watanabe (1936) also believes that the choana opens into the ectoderm, but gives no clear evidence to support his contention. He made his studies on cross-sections only. From my experience such sections are unsuitable for the elucidation of this point. To ascertain whether the choana opens into the ectoderm or endoderm, it is desirable that these two layers be well represented in a given section so that they can be easily compared and one is certain which layer he has under view. For such studies, sagittal sections should be employed. In a cross-section generally only one or the other of these embryonic layers is present, or, when both are included, they are represented by fragments which do not lend themselves to such comparative studies. I have cross-sections which closely resemble the figures in Watanabe's paper but which proved worthless for determining the nature of the choanal opening. It is only after one has familiarized oneself with the character of these two layers by studying sagittal sections that one can tell them apart in cross-sections.

#### SUMMARY.

1. The anlage of the olfactory placode arises from the sensory layer of the ectoderm.
2. There is no oro-nasal groove between the olfactory placode and stomodaeum.
3. The primitive choana opens into the endodermal part of the oral cavity.
4. The lateral appendix is the earliest formed blind sac. It degenerates and disappears at the end of the third stage.
5. There are three blind sacs in the larval stage: the upper, anterior lower, and posterior lower sac. The first and third sacs together become the cavum principale and recessus lateralis, while the second becomes the cavum inferius and recessus medialis in the adult stage.

6. The nasal cavities undergo considerable shrinkage during metamorphosis.

7. Extra blind sacs may be formed in the later larval stage.

8. The naso-lacrymal duct arises in the dorso-lateral part of the cavum principale and extends to the lower eyelid. The process of hollowing out appears to begin from the distal end.

9. There are four kinds of glands in the nasal cavity—medial nasal, lateral nasal, Bowman's, and pharyngeal gland. The first one arises earlier than the other three.

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# Morphological observations on the fate of the Lateral Appendix in the embryonic Olfactory Organ of *Rana nigromaculata*.

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With 12 Text-figures.

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## INTRODUCTION.

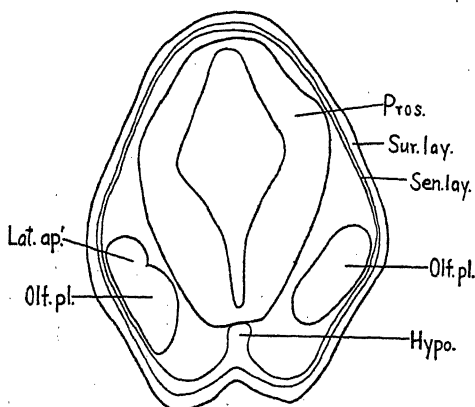
IN the early development of the olfactory organ of the Amphibia there appears an ephemeral structure, the lateral appendix, which persists for a time and degenerates in the later stages of the nasal development. The function of this organ is obscure. The present investigation is to give an account of the structure and development of the lateral appendix as a preparation for later experimental studies. For the development of the whole nasal organ the reader is referred to a previous paper by the writer (Tsui, 1946).

## TECHNIQUES.

The techniques employed in the present investigation have been described in a previous paper (Tsui, 1942). For the purpose of studying the finer structures serial paraffin sections were cut  $5\mu$  in thickness. For every stage three specimens were used, and stained respectively with Delafield's haematoxylin, Mallory's triple stain and Heidenhain's haematoxylin in order to bring out the structure of every part of the cell.

## DEVELOPMENTAL ANATOMY.

The formation of the lateral appendix in *Rana nigromaculata* Hallowell begins at the second stage of development of the olfactory organ. When the larva is 5.5 mm. in length, the olfactory pit is beginning to extend to form the middle lumen and its dorso-lateral part lengthens posteriorly



TEXT-FIG. 1.

Cross-section of the head through the olfactory placode and the anlage of the lateral appendix.  $\times 75$ . *Hypo*, hypophysis; *Latap'*, anlage of lateral appendix; *Olfpl*, olfactory placode; *Pros*, prosencephalon; *Senlay*, sensory layer of ectoderm; *Surlay*, surface layer of ectoderm.

## ABBREVIATIONS FOR TEXT-FIGS. 1-12.

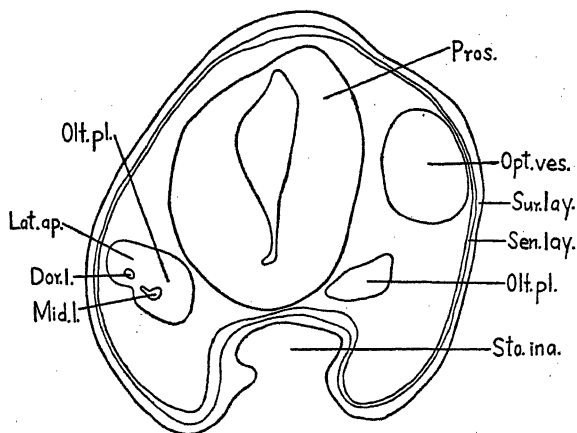
(Text-figs. 7-12 drawn with the aid of a camera lucida.)

*Cil*, cilia; *Cyto*, disintegrated cytoplasm; *Latap*, vestige of the lateral appendix; *Mito*, mitosis; *Nuc*, normal nucleus; *Nuc''*, degenerated nucleus; *Nuc'*, beginning of the degeneration of nucleus; *Olfep*, olfactory epithelium; *Piggr*, pigment granules; *Piggr'*, degenerated pigment granules; *Yogr*, yolk granules.

to form a small tube. The cells directly behind this tube are immediately differentiated into the anlage of the lateral appendix. In the beginning the anlage is not sharply marked off from the olfactory placode as the constriction between it and the placode is now barely perceptible (Text-figs. 1 and 7). The placode is at this time in the form of a thick disc and is still

appressed to the sensory layer of the ectoderm. With the placode in this position, the anlage of the lateral appendix is not yet lateral to it, but is situated on its dorsal part and is posterior to the olfactory pit (Text-figs. 1 and 7).

After the formation of the middle lumen (the length of the larva is now about 7 mm.), the blind end of the small tube enlarges and extends into the anterior part of the anlage of the



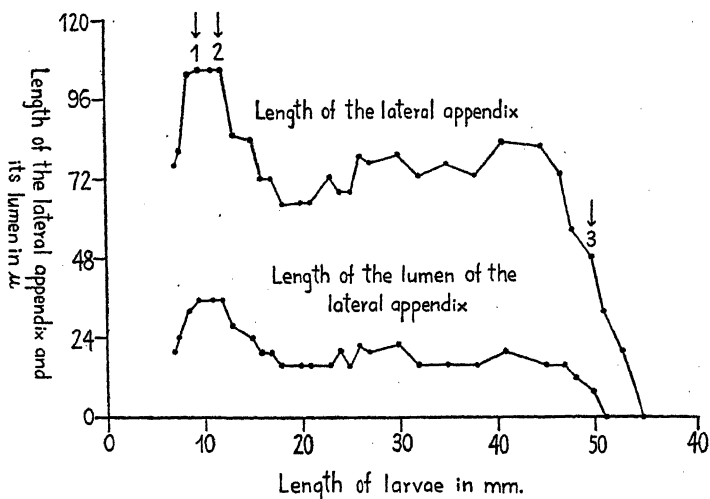
TEXT-FIG. 2.

Cross-section of the head through the olfactory placode and the lateral appendix.  $\times 75$ . *Dorl.*, dorsal lumen; *Latap.*, lateral appendix; *Midl.*, middle lumen; *Optves.*, optic vesicle; *Stoina.*, stomodaeal invagination. For other abbreviations see Text-fig. 1.

lateral appendix, thus converting it into a blind sac. In this way the lateral appendix proper comes into being (Text-figs. 2 and 8). Its length is now about  $76\mu$ , while its lumen, which is termed the dorsal lumen, is about  $20\mu$  (Text-fig. 3). The relative size of the lumen to the lateral appendix is small and remains at the anterior part of the latter throughout its developmental history.

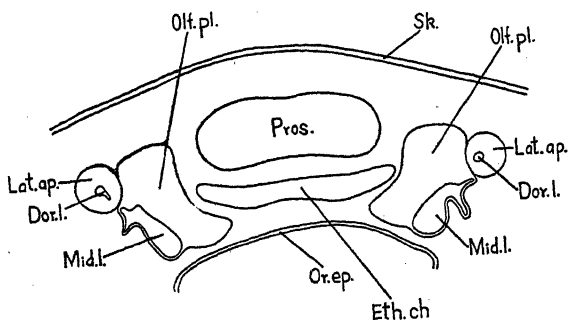
In the course of development, the olfactory placode expands more rapidly at the medial region and thrusts the lateral appendix from its original position to a new one lateral to the





TEXT-FIG. 3.

Graphic representation of the length of the appendix and that of the lumen during growth and degeneration. Arrow 1 indicates the appearance of the anterior lower sac; Arrow 2, appearance of the medial nasal gland; Arrow 3, the beginning of the metamorphosis.

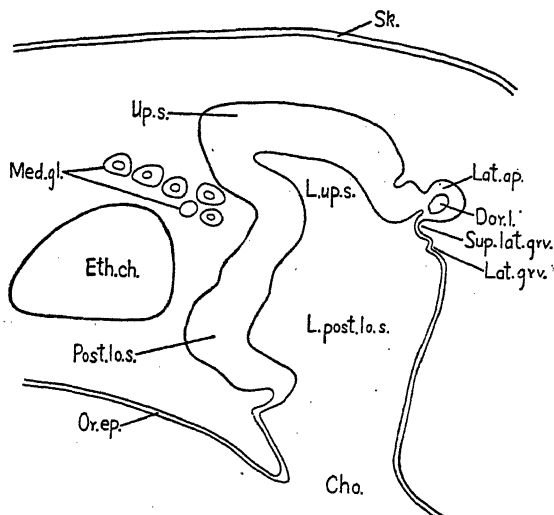


TEXT-FIG. 4.

Cross-section of the upper half of the head through the olfactory placode and the lateral appendix.  $\times 75$ . *Ethch*, ethmoid part of the chondrocranium; *Or. ep.*, oral epithelium; *Sk.*, skin. For other abbreviations see Text-figs. 1 and 2.

placode. A prominent constriction now appears between these two structures (Text-figs. 4 and 9).

Immediately after the formation of the lateral appendix, its posterior end lengthens out hanging free of the placode. The body of the lateral appendix together with its posterior extension increases in dimensions along with growth of the placode



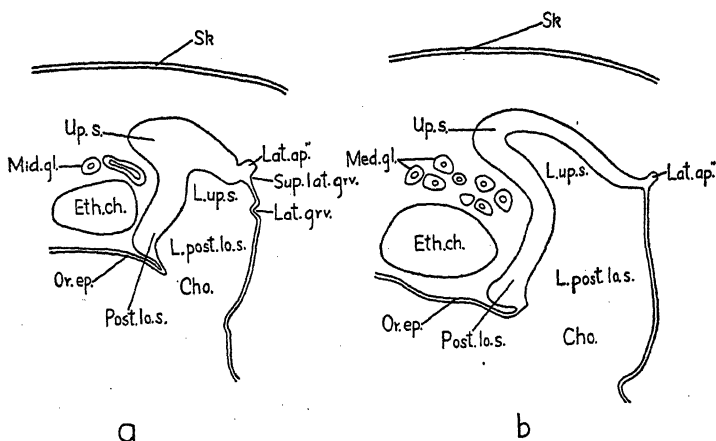
TEXT-FIG. 5.

Cross-section of the right olfactory organ through the lateral appendix and the choana.  $\times 75$ . *Cho*, choana; *Dorl*, dorsal lumen; *Ethch*, ethmoid part of chondrocranium; *Lpostlos*, lumen of posterior lower sac; *Lups*, lumen of upper sac; *Latap*, lateral appendix; *Latgrv*, lateral groove; *Medgl*, medial nasal gland; *Orep*, oral epithelium; *Postlos*, posterior lower sac; *Sk*, skin; *Suplatgrv*, super-lateral groove; *Ups*, upper sac.

up to the beginning of the third developmental stage, when the larva has reached a length of 9.5 mm. The growth of the appendix is then suddenly arrested and remains stationary for a very brief period. After this it begins to degenerate. The easily ascertainable evidence of degeneration is the diminution of its dimensions (Text-fig. 5). The curves in Text-fig. 3 represent the whole life-history of the lateral appendix in the course

of nasal development in terms of its length and the length of its lumen. Their widths both in growth and degeneration correspond with their lengths.

After a sharp decrease, the length of the appendix and its lumen remains again more or less stationary (the fluctuations are greater for the body of the appendix than for the lumen).



TEXT-FIGS. 6 A AND 6 B.

6 A. Cross-section of the right olfactory organ through the lateral appendix and the choana.  $\times 50$ .

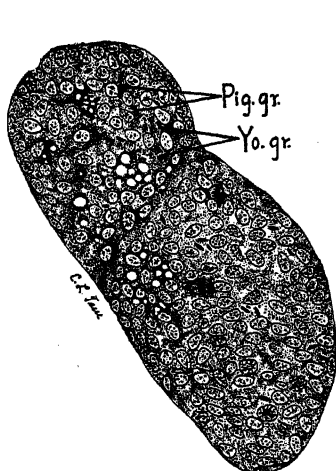
6 B. The same, at a later developmental stage.  $\times 50$ . *Latap*\*, vestige of the lateral appendix. For other abbreviations see Text-fig. 5.

Towards the end there is again a rapid decrease. At the beginning of metamorphosis the lateral appendix is reduced to a very small remnant attached to the lateral posterior part of the upper sac above the super-lateral groove (Text-figs. 6 A and B). Later it disappears completely in the nasal cavity.

The cause of the degeneration of the lateral appendix is not known. It is remarkable, however, that the sudden arrest of its development is just at the beginning of differentiation of the anterior lower sac. Furthermore its degeneration starts at the point when the medial nasal gland makes its appearance.

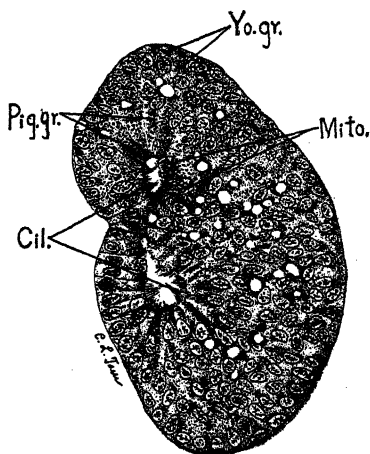
## DEVELOPMENTAL HISTOLOGY.

The anlage of the lateral appendix consists of undifferentiated epithelial cells. Their nuclei are ovoid in shape and are crowded together so that the outline of individual cells cannot be made out. Chromatin granules are comparatively small. Large yolk-granules and numerous fine pigment granules are present in the



TEXT-FIG. 7.

Fig. 7.—Enlarged and detailed view of the left olfactory placode with the anlage of the lateral appendix shown in Text-fig. 1.  $\times 350$ .



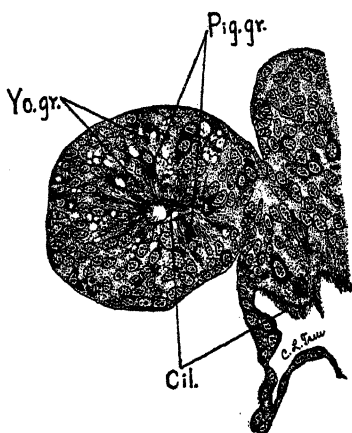
TEXT-FIG. 8.

Fig. 8.—Enlarged and detailed view of the left olfactory placode with the lateral appendix shown in Text-fig. 2.  $\times 350$ .

cytoplasm. The granules are more or less evenly distributed at this stage (Text-fig. 7).

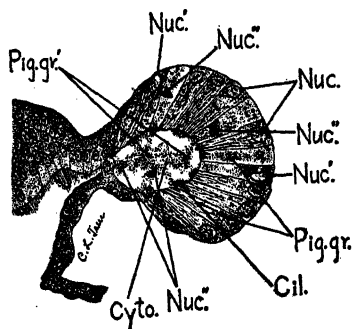
As soon as a blind sac is formed out of this anlage, the cells are differentiated into stratified epithelium containing about four layers of cells at the height of the development of the lateral appendix and about three layers in the earlier stages (Text-figs. 8 and 9). Around the dorsal lumen there is a layer of columnar cells provided with cilia on their free surface. Their nuclei are ellipsoid. The layers of cells in the basal part are not well defined and are represented only by their ovoid nuclei.

Yolk and pigment granules are still present in the cytoplasm; the former decrease in quantity in the course of development, and are completely absorbed when the lateral appendix reaches its full growth. The distribution of pigment granules is no longer so even as in the anlage. A large number of these granules become concentrated in the neighbourhood of some of the nuclei, their aggregate assuming more or less the form of



TEXT-FIG. 9.

Fig. 9.—Enlarged and detailed view of the left lateral appendix with a part of the olfactory placode shown in Text-fig. 4.  $\times 350$ .



TEXT-FIG. 10.

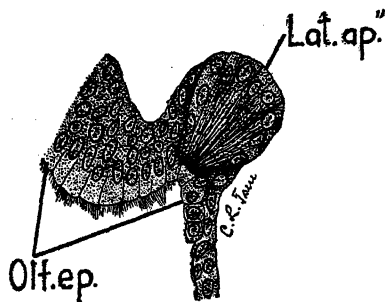
Fig. 10.—Enlarged and detailed view of the lateral appendix with a part of the upper sac shown in Text-fig. 5.  $\times 350$ .

a spindle with the nucleus in the middle. Such aggregates are especially prominent in the advanced developmental stages (Text-fig. 9). Mitotic figures are often seen in the early stages but not later (Text-fig. 8).

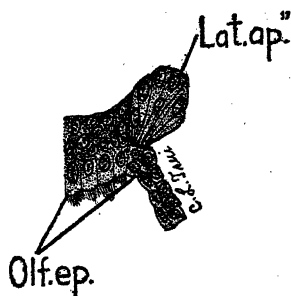
As long as the lateral appendix remains stationary, its microscopic anatomy is as described above, but structural changes immediately take place when it begins to degenerate. The most easily detectable change is the diminution in size. Histologically, this is effected at first by the reduction in the number of cell layers. As described above, the fully developed appendix consists of about four layers of cells. These layers

gradually diminish through the destruction of their cells. In the dorsal lumen or somewhere near it are often found small amounts of remnants of nuclei and cytoplasm, as well as pigment granules and cilia. The stages in the process of degeneration cannot be made out, however. They are probably obscured by the crowded condition of the basal nuclei.

At last the degenerated appendix consists of only one layer of well-defined cells. In this greatly simplified picture, the disintegration of various elements are more easily observable. This



TEXT-FIG. 11.



TEXT-FIG. 12.

Fig. 11.—Enlarged and detailed view of the vestige of lateral appendix shown in Text-fig. 6 A.  $\times 190$ .

Fig. 12.—The same shown in Text-fig. 6 B.  $\times 190$ .

last layer of cells is highly columnar (Text-fig. 10). Their nuclei are ovoid in shape and are always situated at the basal part of the cells. The cytoplasm is fibrillar and takes the stain very faintly. The free surface of most of the cells is devoid of cilia, or, when they are present, they are scanty and appear stuck together. In most cases, the lumen is filled with disintegrated cytoplasm containing fragments of nuclei, cilia, and pigment granules. Sometimes the lumen is so filled with debris that the surrounding columnar cells are flattened and become squamous in shape.

The columnar cells as described in the preceding paragraph appear still normal under the microscope. When their degeneration is evident, their nuclei become kidney- or horseshoe-

shaped. The chromatin granules are very scarce and take the stain faintly. They finally disintegrate. Their cytoplasm loses the fibrillar structure and appears disorganized. Cilia are totally absent. The pigment granules are few and scattered, and are finally discharged into the lumen (Text-fig. 10).

As the olfactory organ further develops, the degeneration of the lateral appendix continues. At its last stage, its lumen becomes obliterated, and the remainder of its cells become partially embedded in the olfactory epithelium as a small vestige (Text-fig. 11). The last trace of it (Text-fig. 12) disappears completely towards the end of the metamorphosis.

#### DISCUSSION.

The role which the lateral appendix plays in the embryonic olfactory organ in *Rana nigromaculata* is not clear. The histological structure of the appendix is similar to the early undifferentiated olfactory placode. Each possesses a lumen lined with cells which possess cilia. They are probably alike in function. The presence of the lateral appendix may have the advantage that the inhalant water can make a considerably longer circuit and thus react with a greater surface of the olfactory epithelium.

The development of the lateral appendix is suddenly arrested at the appearance of the anterior lower sac and it starts to degenerate after the formation of the medial nasal gland. This synchronism is probably more than a coincidence. The portion of the anterior lower sac connected with the medial nasal gland together with the gland develops into the recessus medialis in the adult stage (Tsui, 1946). This structure is identified by Mihalkowicz (1898) as the vomero-nasal or Jacobson's organ in the nose of the Amphibia. This view has been accepted by most anatomists. There have been researches to ascertain the function of this vomero-nasal organ. Broman (1920) made various experiments with reptiles and mammals and came to the conclusion that it is an aquatic organ of smell (Wassergeruchsorgan) which persists in the nose of terrestrial vertebrates. Kerkhoff (1924) repeated Broman's experiments on the horse and came to the same conclusion. Observations of

the recessus medialis or vomero-nasal organ reveal that it contains more olfactory cells than any other part of the nasal cavity. The lumen of it is always filled with the secretion from the medial nasal gland. It is fairly certain that it is an aquatic organ of smell in the Amphibia. Why does the lateral appendix degenerate when the beginnings of the recessus medialis appear? A plausible view is that the lateral appendix is a forerunner of the vomero-nasal organ in early life of the Amphibia, and is soon replaced by the latter organ. It is proposed to conduct experimental studies to test the validity of this view.

#### SUMMARY.

1. The anlage of the lateral appendix is formed from the dorso-lateral part of the olfactory placode in *Rana nigromaculata* Hallowell.

2. The lateral appendix, a blind sac, is formed when the dorsal lumen is differentiated in its anterior part.

3. It increases in dimension as the olfactory placode further develops, but growth stops as soon as the anterior lower sac appears; it begins to degenerate when the medial nasal gland makes its appearance; and completely disappears towards the end of metamorphosis.

4. Histological changes are described.

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# The Morphology and Relations of the Siphonophora.

By

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'Anyone who has studied the history of science knows that almost every great step therein has been made by the 'anticipation of Nature', that is, by the invention of hypotheses, which, though verifiable, often had very little foundation to start with; and, not infrequently, in spite of a long career of usefulness, turned out to be wholly erroneous in the long run.'

T. H. HUXLEY: 'The Progress of Science' (1887).

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With 57 Text-figures.

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## INTRODUCTORY.

WISHING recently to put together the evidence concerning the origin of the Pelagic Fauna, I found, when I came to the Siphonophora, that their morphology was so dominated by

dubious theories under the aegis of great names that a review of the literature would be necessary to disentangle fact from fiction. The present paper is an outcome of that review, and contains some remarkable examples of the persistence of doctrines long after their foundations had disappeared.

Following their classical investigations of Siphonophore development, Haeckel (1869) and Metschnikoff (1874) both came to regard Siphonophores as transformed Medusæ, the great larval bract of *Physophora* being interpreted as a split and reduced umbrella and the polyp as its manubrium—a view which even the judicious Balfour (1885) approved. To this Metschnikoff, protagonist of the ‘poly-organ’ theory, added the idea that the float itself was a second umbrella, but retroverted, like the domestic article turned inside out, as a gas-holder. Both were impressed by the budding powers of the manubrium in various species of *Sarsia*, and Haeckel built upon them his elaborate ‘medusome’ theory, according to which all the zooids of a Siphonanth colony were interpreted as dislocated parts of so many dismembered medusoid buds of a proliferating Anthomedusan. But he treated the Disconanths differently. A *Veilella* or a *Porpita* was not a colony of medusomes, but a single Trachomedusan, of which the manubrium alone has been multiplied on the subumbral surface. To these medusa-based speculations of the ‘heroic age’ have since been added the ‘Bipolaria’ hypothesis of Woltereck (1905) and the ‘Heteromedusa’ theory of Moser (1925). All imply a pelagic ancestry of the group, and the last is as recent as the latest textbook.

On the other hand adherents to the earlier view of Leuckart and Vogt (1848–54), that Siphonophores are simply Hydro-medusan colonies adapted by further polymorphism to a pelagic life, have not been wanting (e.g. Claus, Chun, Schneider), though their advocacy has scarcely advanced their cause. Rejecting Metschnikoff’s interpretation of the float as an inverted umbrella, Leuckart himself claimed (1875) that the initial invagination which gives rise to it may be identified with the entocodon or *Glockenkern* of a medusa bud; Claus (1878) followed this up by homologizing the pericystic spaces between the radial septa of *Physonecks* with the radial canals of a medusa; and Chun

(1897 *a*) completed the argument by identifying this medusa with the primary nectocalyx of the Calycophore cycle. This view of the pneumatophore as a modified nectocalyx is the view now generally held, except for a minor qualification by Woltereck and Moser, that it should be homologized with the second or 'dorsal' bell of Calycophora, and not with the first or larval bell, which is caducous. It will be noted that this theory, while preserving the homology of the larva with a Hydromedusan Planula, sets up the Calycophore as the primitive type of Siphonophore, and thus indirectly leads to much the same result as the previous 'medusoid' theories, viz. the derivation of Siphonophora from an actively swimming original stock, one, however, which, in its organization as a whole, appears to be most remote from the Hydromedusan type.

It will be convenient to deal with this special proposition at the outset, for it lies at the base of Siphonophore morphology, and its widespread acceptance makes it the more serious. In the last half-century Haeckel has been its only outstanding opponent, for, although holding the Siphonophore larva to be a transformed medusa, he refused to see anything more in the float than an invaginated aboral gland. In this, as we shall see, he was probably right.

#### SUMMARY OF CHAPTERS.

1. DEVELOPMENT OF THE PNEUMATOPHORE.—The float is developed from a simple apical invagination without an entocodon and shows no traces of origin from a medusa or nectocalyx. Its 'radial septa', when present, result from a secondary protrusion of giant cells from the gas gland as 'hypocystic villi', which may, or may not, fuse with the outer endoderm of the pericystic coelenteron. (P. 109.)

2. CALYCOPHORE AND PHYSOPHORE.—In Calycophora the aboral extremity of the larva atrophies, so that the pneumatophore has no homologue in this group, medusoid or otherwise. The primary nectocalyx is a ventral bud which secondarily assumes a subapical position. There is no 'aboral manubrium'. (P. 117.)

3. DISCONANTH AND SIPHONANTH.—Haeckel's distinction is valid, and independent of his phylogenetic theories. The former type is radially symmetrical, with an aboral whorl of simple tentacles, and developed from an Actinula larva (Conaria): the latter, bilaterally symmetrical, with a separate basal tentacle to each polyp, and developed from a solid Planula larva by unilateral (ventral) budding. (P. 118.)

4. THE HYDROID RELATIONS OF DISCONANTHÆ.—These show close relationship with Tubularians, especially *Corymorpha*, in the dominance of a large axial polyp with plexiform aboral coelenteron, aboral wreath of tentacles, free Anthomedusan gonophores, and an *Actinula* larva. (P. 123.)

5. CONARIA AND THE CORYMORPHINES.—Woltereck's larva of *Velella* (*Conaria*) has a complete gastric diaphragm, homologous with that of *Corymorphine* Hydroids, but modified as a larval organ of flotation secreting oil-drops. Its aboral chamber gives origin to the primary radial canals and plexus around the float, as that of *Corymorphines* does to the branching canals of the stalk. (P. 127.)

6. THE SIPHONANTH PROBLEM.—The differences from *Disconanth*s are reviewed. Although the actual larvae of *Siphonanth*s are *Planulæ*, they develop from large yolky eggs, usually one in each gonophore like *Actinulæ*, and begin to bud with extraordinary precocity, so that original *Actinuloid* characters may have been suppressed or retarded. (P. 133.)

7. GASTRULATION AND THE BUDDING LINE.—The precocity of *Siphonanth* budding associates it with the meridian of first gastrulation, which proceeds slowly up each side from venter to dorsum. This initial budding line is made permanent by development of a thick muscular layer everywhere else, which is prohibitive of radial budding. (P. 135.)

8. THE NATURE AND ORIGIN OF BRACTS.—These early appendages are regarded as larval tentacles primarily adapted for locomotion—a view supported by particular life-histories and by reference to *Pelagohydra* and the multi-tentaculate *Actinula* of *Myriothela*. (P. 139.)

9. NECTOSOME AND SIPHOSOME.—The distinction of these regions, with a budding zone between them, corresponds with that of hydrocaulus and hydranths in a *Hydroid* (cf. *Pelagohydra*). The cormidia correspond to the trimorphic branches of *Dicoryne*, with terminal polyp, blastostyle, and gonophores. The chief difference between them is in their arrangement: perpetual forking in *Hydroids*, a linear succession in *Macrostelia*, and the formation of homogeneous whorls in *Brachystelia*. (P. 146.)

10. CORMIDIAL BUDDING IN MACROSTELIA.—The buds of each polymorphic cormidium arise as a group (in *Calycophora* from a single pro-bud), and are carried backwards as fast as they are produced by a pronounced longitudinal growth of the oozoid in the zone of proliferation. This 'metameric' attenuation is no primitive feature, but a simple adaptation for better fishing: it extends the slender fishing tentacles (one to each cormidium) through a greater range of water. (P. 150.)

11. GROWTH AND SYMMETRY IN BRACHYSTELIA.—The oozoid remains short, and in contrast with the *Macrostelia*, exhibits, especially in the siphosome, an excess of horizontal over longitudinal growth. This carries the buds of each cormidium from the ventral line successively towards the dorsum, with the resultant formation of homogeneous parallel or concentric whorls. This growth may proceed (i) with complete bilateral symmetry, as

in Anthophysa and in the nectosome of Rhodaliidae, or (ii) asymmetrically from one side only, as in the siphosome of Physophora, a condition which leads to (iii) the continuous cormidial spires of Discolabe and the Rhodaliids. Though imperfectly known, there is reason to expect that Epibulia may be found to be perfectly biradial, like the Corymorphine Branchiocerianthus. The bracts of Nectalia are not cormidial but coronal, and relate this genus to Anthophysa rather than to the Agalmids. (P. 155.)

12. GENERAL CONCLUSIONS.—The gap between Disconanths and Siphonanth is materially reduced, but not bridged, by these results. On present knowledge it would appear that Disconanths are especially related to Tubularians, and Siphonanth to Corynoids; but the structure of Pelagohydra and Myriothela suggest the possibility of a common origin from some intermediate type of Gymnoblast which gave rise to both stocks. A settlement of this issue can hardly be expected without additional knowledge on various points, especially the development of Myriothela and Corymorpha. (P. 175.)

13. SYSTEMATIC.—The results of the present study are expressed in a revised classification based on Eschscholtz's tripartite division into Chondrophorae, Physophorae, and Calycophorae, and in that order, which reverses Chun's system; but it is combined with a retention of Haeckel's major groups to mark the gap between Chondrophorae and the remainder. Pending the establishment of definite lineages, Haeckel's terms Brachystelia and Macrostelia are also used in each sub-order of Physophorida as descriptive terms, the long-bodied forms having plainly arisen at least twice independently from the more archaic Brachystelia. The term 'Amphinecta' is proposed as a substitute for Haeckel's 'Physonectae'. (P. 189.)

## GLOSSARY OF TERMS.

(For systematic names see Revised System, § 13, p. 189.)

**ACTINULA.**—Hydrozoan larva with precocious coelenteron, mouth and tentacles.

**BASIGASTER.**—Basal region of a Siphonanth polyp, packed with young cnidoblasts, and giving off the tentacle.

**BLASTOSTYLE.**—See GONOPHORE. Often wrongly used in Chondrophora.

**BLASTOZOOID.**—A zooid budded from the primary individual or oozoid.

**BRACHYSTELIA.**—Descriptive term for short-stemmed Physophores.

**BRACT** or **Hydrophyllium.**—An appendage of Siphonanth with a narrow blind, axial canal and much mesogloea, variously modified.

**CENTRADENIA.**—The 'central gland' or organ of Chondrophora, derived from the ectoderm, lodged in the mesogastric septum, packed with young cnidoblasts and eventually traversed by endodermal canals.

**CORMIDIUM.**—A polymorphic cluster of blastozooids in Siphonantha, including a gonodendron or gonophore and a siphon, with or without one or more bracts and palpons.

**CORONA.**—A ring of uniform appendages or zooids around the nectosome.

**DIAPHRAGM.**—The iris-like annular septum which divides the gastric cavity of *Corymorphine* polyps.

**ENDOCHORD.**—Name here given to the solid endodermal axis, surrounded by a longitudinal plexus of canals, in the *Tubularian* stalk.

**ENTOCODON** or *Glockenkern*.—The terminal proliferation of ectoderm in a medusoid bud which eventually hollows out to form the bell-cavity.

**EUDOXIA.**—The free-swimming cormidium of certain *Calycophores*, which consists of a bract with phyllocyst, a gonophore, and a tentaculate polyp, with or without a special nectocalyx.

**GONOPHORES.**—The production of these in *Hydrozoan* colonies is associated with various stages of gastro-genital differentiation, and differences of nomenclature are linked with each stage. Those which most concern us are the following:

- (a) Oozoid (?) and all blastozooids fully polypoid, and fertile in the sense that each gives rise directly to sexual medusoid gonophores. Most arborescent *Gymnoblasts*.
- (b) Oozoid itself sterile, but giving rise by budding to complete and fertile **SECONDARY POLYPS**, which directly produce medusoid gonophores. *Chondrophora*.
- (c) Oozoid sterile; blastozooids differentiated into sterile *Gastrozooids* (fully formed **POLYPS**) and fertile mouthless *Gonozooids* (**BLASTOSTYLES**) which alone produce gonophores. *Dicoryne*. *Myriothela*. *Pelagohydra*?
- (d) As (c), but the sterile *Gastrozooids* are further differentiated into 'Siphons' with mouth and 'Palpons' mouthless; and the *Gonozooids* (**GONOPALPONS**), by branching, give rise to dense clusters of gonophores, together known as **GONODENDRA**. These again may be divided into male branches, forming *Androphores*, and female branches, producing *Gynophores*. *Physophorida*.

In *Calycophorida* gonophores are produced in limited numbers and separate blastostyles (gonopalpons) have apparently disappeared.

**HYPOCYSTIC.**—i.e. below the pneumatocyst.

**MACROSTELIA.**—Descriptive term for long-stemmed *Physophores*.

**NECTOCALYX** or *Nectophore*.—An asexual medusoid gonophore specialized for locomotion of the colony.

**NECTOSOME.**—Aboral region of oozoid bearing the float and/or the locomotive bracts or nectocalyces. Opposed to *Siphosome*.

**Oozoid.**—The primary individual or zooid produced from an egg, and itself producing additional zooids (blastozooids) by budding.

**PALPON.**—A *Siphonanth* polyp arrested in development at the mouthless stage and specialized. Its tentacle ('palpacle') may persist (e.g. *Physophora*) or be suppressed (*Epibulia*). One form (gonopalpon) is the *Siphonanth* blastostyle.

**PERICYSTIC.**—i.e. around the pneumatocyst.

**PHYLLOCYST.**—The dilated axial canal of a cormidial bract, usually laden with oil-drops and serving as a float (Calycophora).

**PLANULA.**—The early larva of primitive Hydroids and of Siphonanthus, lacking mouth and appendages, and solidly filled with yolk-endoderm.

**PNEUMATOCYST.**—The chitinous 'float' of Siphonophora, secreted by an invaginated sac of the apical body-wall.

**PNEUMATOPHORE.**—The whole aboral extremity moulded around the enclosed float. It consists of three parts: the outer body-wall (pneumatocodon), the invaginated body-wall (pneumatossaccus), and the pericystic coelenteron between them.

**PROTOSIPHON.**—The gastric region (hydranth) of the primary polyp or oozoid.

**SIPHON.**—The gastric region (hydranth) of a polyp reproduced in blastogenesis; hence almost synonymous with polyp.

**SIPHOSOME.**—The gastro-genital hind-body, composed of the hydranth of the oozoid (primary polyp) together with its budding zone and the blastozooids produced from it, which constitute the cormidia. Opposed to Nectosoma.

**SOMATOCYST.**—The endodermal outgrowth with surrounding mesogloea at the base of a developing nectocalyx in Calycophora. It becomes usually filled with sappy cells secreting oil-drops, and corresponds to the phyllocyst of a cormidial bract. Its inclusion in the nectocalyx implies the incorporation of a bract, Schneider's 'bract-bell'.

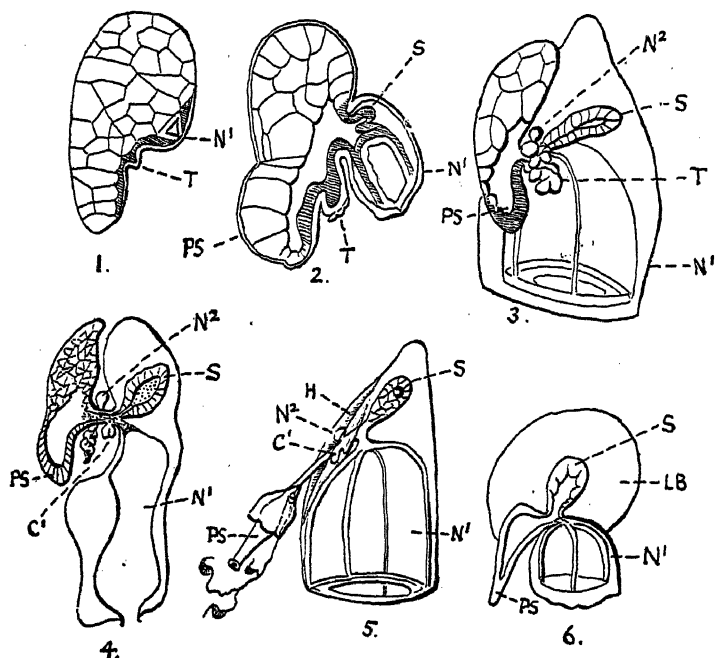
**STEM.**—The axial foundation of a Siphonophore colony, i.e. the mid-body of the oozoid from which the buds arise. By intermittent elongation it produces the metamerized colonies of Macrostelium; by intermittent growth in width it carries the cormidial buds dorsally, thus producing the homogeneous whorls of Brachystelia.

## 1. DEVELOPMENT OF THE PNEUMATOPHORE

(with Text-figs. 1-11).

Omitting earlier history, of which excellent summaries have been given by Schneider (1896) and Chun (1897 a), I take Chun's illuminating article 'Ueber den Bau und die morph. Auffassung der Siphonophoren' (1897 a) as giving mature expression to the case for regarding the pneumatophore as the homologue of a nectocalyx. It rests on three propositions: (a) that its position is comparable with that of the primary nectocalyx of the Calycophore, (b) that the 'thickened ectodermal invagination' from which it arises represents the Glockenkern of a nectocalyx, and (c) that it is surrounded (with certain





TEXT-FIGS. 1-6.

Development of Calycophorida, showing atrophy of aboral region of oozoid and composite nature of primary nectocalyx. *C*, cormidium; *H*, hydroecium; *LB*, larval bract; *N*, nectocalyx; *PS*, protosiphon; *S*, somatocyst (= endoderm of larval bract); *T*, tentacle.

Figs. 1, 2, 3.—*Galeolaria quadrivalvis* (Metschnikoff, 1874).

Figs. 4, 5.—The same, after Lochmann, 1914. Fig. 4 in sagittal section.

Fig. 6.—Metschnikoff's larva of '*Praya inermis*?' (probably *Sphaeronectes truncata*), showing incomplete fusion of larval bract (*LB*) with nectocalyx (*N*). Cf. fig. 12.

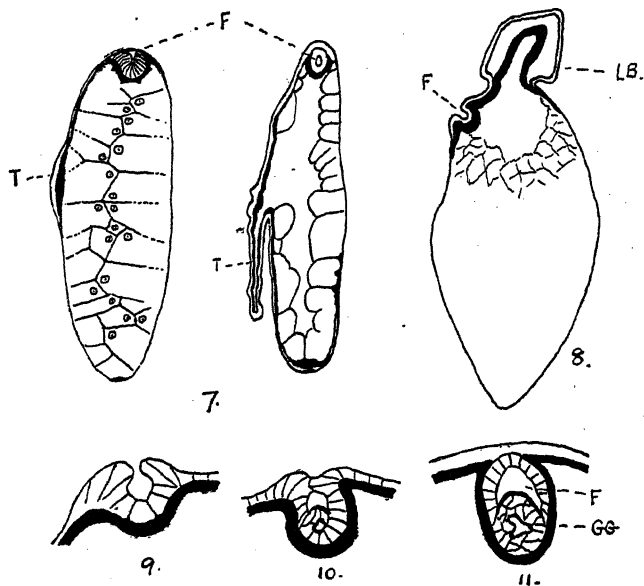
exceptions) by a series of radial cavities of the coelenteron which are homologous with the radial canals of the nectocalyx.

(a) The first of these propositions is largely a matter of judgment, for in some of the more yolky Agalmid larvae, which retain the spherical form of the embryo, exact determination of the apical pole is wellnigh impossible; but if the comparison is limited to the more elongated types of larvae (e.g. *Stephanomia picta*, *Physophora*, *Galeolaria*, *Muggiaea*) (Text-figs. 1-11), then there can be no doubt that, while in *Physophores* the rudiment of the pneumatophore is truly apical, that of the primary nectocalyx of *Calycophores* is definitely ventral. This discrepancy of position is confirmed on the one hand by the strictly median and aboral position of the pneumatophore invagination in the larva of *Velella* (Text-figs. 15-24), and on the other by the fact that in larval *Calycophores* the aboral region of the larval body remains stuffed with yolk during the critical stages of development, and is finally absorbed without having contributed anything to the formation of the nectocalyx.

The atrophy of the aboral half of the larval body in *Calycophores* was described seventy years ago with full illustrations by Metschnikoff (1874, Pls. vi, vii), after rearing the eggs of *Galeolaria quadrivalvis* (his '*Epibulia auran-tiaca*'), and isolated stages of other species have been described which show it to be a general feature. The sequence was confirmed in all respects thirty years ago by Lochmann (1914), who reared the same species to an even later stage. The failure of all the experts to realize its significance is truly astonishing. As only the early stages of Metschnikoff's series have been figured in the textbooks I here give copies of later figures from the two papers mentioned (Text-figs. 3 and 4).

Moser's proposal (1924, 1925) to homologize the pneumatophore with the definitive or so-called 'dorsal' nectocalyx, instead of the larval bell, is equally invalid, for this second bell develops as a bud from the stalk of the first, and is therefore also of ventral origin.

(b) The second proposition was based on Metschnikoff's early accounts already cited (1874), and the relevant figures of



TEXT-FIGS. 7-11.

Physophorida, showing development of float in Agalmids. Endoderm black in 8-11. *F*, float; *GG*, gas-gland; *LB*, larval bract; *T*, tentacle.

Fig. 7.—*Stephanomia picta*, two stages (Chun, 1897).

Fig. 8.—*Halistemma rubrum* (Woltereck, 1905).

Figs. 9, 10, 11.—Successive stages in *Agalma elegans* (Woltereck, 1905).

*Galeolaria* (his 'Epibulia') and *Stephanomia* (*Halistemma*) are in the textbooks. But these were impressions from surface views or optical sections, and in 1897 Chun figured the earliest stage of the pneumatophore in the same *Physophore* (*Halistemma pictum*) as a shallow open invagination which subsequently closed (1897, fig. 15: my Text-figs. 7-11), and in 1905 Woltereck, from sections demonstrated to the German Zoological Society, figured the rudiments of the float as open invaginations, both in the *Actinula* larva ('*Conaria*') of *Velella* (1904) and in the Planulae of *Agalma elegans* and *Halistemma rubrum* (1905 b). There is, therefore, nothing in the early rudiment of the float to point specifically to a medusoid origin, even though Chun and Woltereck still clung to that idea. All sorts of organs have developed from ectodermal pits, and the demonstration in this case of the absence of any entocodon-like proliferation removes the only feature that might be regarded as demonstrative.

In her 'Gauss' Report, as well as in her article in Kükenthal's great 'Handbuch', Dr. Fanny Moser states that a 'Glockenkern' is absent in the development of most Calycophore bells, whether gonophores or nectocalyces, while all bells of Physophores (including the pneumatophore!) pass through a Glockenkern stage. I have been unable to find upon what evidence she based her statement about Calycophora which, if correct, would be of much interest in connexion with the problem of ovogenesis in that group; but in any case the absence of an entocodon would not affect my point that a simple open invagination of ectoderm is no proof of a medusoid origin. It is also far from supporting the extraordinary view she maintains that the absence of a Glockenkern in Calycophores is a more primitive feature than its presence in Physophores!

(c) A similar lack of cogency applies to the arguments based on the so-called 'radial pouches' of the pericystic region of the coelenteron (Claus, 1878; Chun, 1887, 1897 a). These spaces vary greatly in number according to size and age, and are very rarely limited to the number (4) of the radial canals. They do not diverge from a common stalk-canal, as do the radial canals of a medusa bud (for there is no 'stalk' to contain one!), but

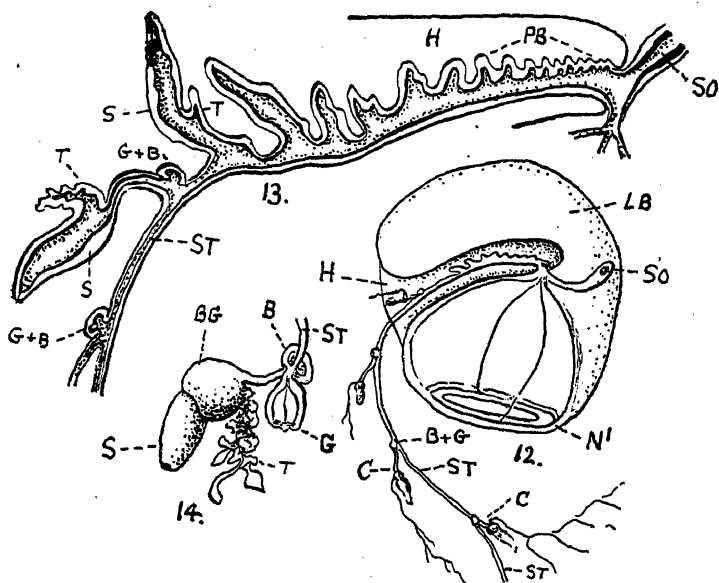
proceed directly and independently from the axial gastral cavity. They are in fact not 'pouches' from this cavity, but parts of it. Referring to Chun's figure of the relations in *Physophora* (1897 *a*, fig. 16), it will be seen that it is not the 'pouches' that need consideration, but the radiating septa which divide and create them. Far more appropriate than the comparison with Medusan canals was that with Actinian mesenteries which was drawn by their discoverer, Milne-Edwards, when describing them 100 years ago: 'The air-vesicle [of *Forskalia*] is . . . retained in a central position by membranous partitions disposed in a radiating manner, and stretched between its parietes and those of the great pyriform cavity (of the upper end of the stem) nearly in the same way as the mesenteries by which the alimentary canal is surrounded in the Alcyonarian polyps' (quoted by Huxley, 1859, p. 6). But the septa of the pneumatophore cannot be regarded as primarily for the support of the air-sac, since they are absent in *Cystonectae*, where the pneumatophore and air-sac are most voluminous; they do not grow inwards from the outer wall, like those of Actinians, but outwards from the wall of the air-vesicle, and each encloses one of the remarkable rows of ectodermal giant-cells, which project outwards as rootlets from the gas-gland of the air-sac.

In *Cystonectae* neither pouches nor septa are present, but conspicuous tufts of these giant-cells protrude freely as 'hypocystic villi' from the air-sac into the undivided pericystic space, each clothed with the ciliated endodermal epithelium which they have pushed before them (Chun on *Rhizophysa*, l.c., fig. 17; Huxley, l.c., figs. 14 and 15; Haeckel, 1888, Pl. xxiv). In *Anthophysa* (Text-fig. 45)—usually classified under *Physonectae*, but in many ways a connecting link with *Cystonects*—which Chun described as possessing free upstanding villi (l.c., fig. 20), it has since been shown by Bedot (1904) and Bigelow (1911, Pl. xxi) that, while most of the villi are free, those on the dorsal side extend across the pericystic space, where their endodermal coat fuses with the outer endoderm, thus forming true septa. Finally, in *Physophora* and the *Agalmids* all the septa are complete, and all contain their axial core of giant-cells. Even in *Rhodaliids* (Haeckel's '*Auronectae*'), in which the

secretory funnel ('aurophore') of the air-sac has undergone its strange displacement, the septa, though irregular, have accompanied the funnel, and conspicuous giant-cells have been recognized in them (Lens and Van Riemsdijk, 1908, in *Archangelopsis*; Bigelow, 1911, in *Dromalia*).

Thus in no Siphonanth is there any real resemblance between the radial pouches and medusoid canals. In the one group (Cystonectae) in which the air-sac retains a functional apical pore (i.e. the bell-mouth of its supposed medusoid predecessor), and should *ex hypothesi* be most medusiform, radial pouches are completely lacking. They are also absent in *Apoemia*, which has been widely claimed as the most primitive Physophore (Chun, 1887). On the other hand there are excellent grounds for holding that the radial septa, when present, have been specially produced within the group by the outgrowth of hypocystic villi from the gas-gland, followed by fusion with the outer endoderm across the pericystic space. Successive stages of this development are exhibited by the sequence Cystonects—Anthophysids—Physonects—the very reverse of the systematic sequence that has been built up on the medusoid hypothesis.

Summing up, I submit that the air-sac of Physophorida is not a transformed nectocalyx, but an independent differentiation of the aboral end of the larval body. It has no counterpart in the Calycophorida in which the aboral region shrivels up without producing anything but yolk. Accurate methods of investigation have shown that the supposed development of the air-sac through an entocodon-stage or *Glockenkern* does not take place. Not a scrap of evidence remains to suggest that the pneumatophore is a medusoid bud: it never starts as a bud, but as a simple involution of the aboral extremity, and the pericystic radial septa which subsequently surround it in one group, are secondary additions to it, entailed by the development of specialized processes from the gas-gland. If Chun and Bigelow are right, as they appear to be, in identifying Haeckel's '*Cystalia*' (l.c., Pl. xxii, 5) with a young stage of *Epibulia* (fig. 6), the hypocystic villi in Cystonects do not project until long after the pneumatophore is well established.



TEXT-FIGS. 12-14.

Calycophorida. Development of cormidia in *Sphaeronectes*.

*BG*, basigaster; *H*, hydroecium, between larval bract (*LB*) and nectocalyx (*N*); *PB*, probuds of cormidia; *SO*, somatocyst; *ST*, stem.

Fig. 12.—Mature colony, stem curtailed. (Chun, 1897 *a*). Cf. Text-fig. 6.

Fig. 13.—Basal portion of stem, enlarged. (Chun, l.c.)

Fig. 14.—Terminal cormidium, with bract (*B*), gonophore (*G*), siphon (*S*), and tentacle (*T*). (Schneider, 1896.)

## 2. CALYCOPHORE AND PHYSOPHORE (with Text-figs. 12-14).

The doctrine of the medusoid nature of the pneumatophore resulted in a general acceptance of the idea that Calycophores were the primitive, and Physophores the derivative stock of Siphonophora, so that the little *Sphaeronectes* (Text-figs. 12-14), as the simplest Calycophore, has been frequently cited as the nearest approach to an archetype of the whole order. Its structure has been summed up as a gelatinous nectocalyx with an exumbral manubrium, which eventually elongates to form the stolo prolifer.

This seemed to confirm a quasi-medusoid theory of the larval forms, since, with the pneumatophore of the one group homologous with, and derived from the primary nectocalyx of the other, a common larval type could be set up, consisting of a medusa at one end, a polyp at the other, and ultimately a stolo prolifer between the two, exactly as in *Sphaeronectes*.

These ideas, doubtless inspired by Woltereck's speculations (1905; cf. p. 129), dominate Dr. Fanny Moser's treatment of the group in the latest of German textbooks, in an article which so far ignores the bounds of fact and fancy as even to define the Siphonophora as 'Proliferating, bilaterally symmetrical Medusae (Heteromedusae) with exumbral (aboral) manubrium'. (Küken-thal's 'Handbuch der Zoologie', the character of which its founder had declared 'auf dem realen Boden der Tatsachen stehen soll!')

As a steady, if not very effective, supporter of the Hydro-medusan theory, Chun (l.c., p. 108) recognised the 'difficulty' of the abnormal position of the manubrium. It never occurred to him that the anomaly might be due simply to the mistake of homologizing an aboral pneumatophore with a ventral nectocalyx. If this homology be set aside, and due recognition given to the atrophy of the aboral region of the Calycophore larva, the 'exumbral manubrium' of *Sphaeronectes* and the larval forms disappears at once. The 'manubrium' of both is the original larval body, secondarily shortened in the Calycophore, in which, in spite of the great disparity in size, it is the little larval body or polyp which bears the gigantic nectocalyx as a



ventral bud—not the nectocalyx which produces an exumbra! manubrium (cf. Text-figs. 2 and 3).

It may be as well here to quote from Metschnikoff's original account: 'In the course of the 7th and 8th days the nectocalyx enlarges to such an extent that all other parts of the larval body appear as mere appendages of it.' 'In further development' the shrinking aboral region 'in which the yolky cells remain longest verwandelt sich in den obersten Theil des Magens' (1874, p. 43).

It follows that it is the Physophore which most fully retains the primitive condition, and the Calycophore which has undergone the most radical change. It is even possible that the atrophy of the aboral extremity of the Calycophore larval body may imply the previous possession of an aboral float, which has been discarded in favour of a precocious nectocalyx.

In any case the facts now appear to lend support to Korschelt and Heider's view of the general direction of Siphonophore evolution, i.e. from passive flotation to active swimming, rather than the reverse, as was implied in all the medusoid hypotheses we have seen reason to reject. This view is in harmony with the evidence of pneumatophore development, which reveals an evolutionary line from Cystonects with a large float and no nectocalyces to Physonects with nectocalyces and a small float, culminating, as we now suggest, in the Calycophore type with nectocalyces and no float at all.

### 3. DISCONANTH AND SIPHONANTH (with Text-figs. 15–16).

Haeckel's distinction between these main sections of the Siphonophora was so mixed up with phylogenetic speculations that the solid grounds for their separation have been largely overlooked. His definitions, couched in terms of his medusoid hypotheses, involved a Trachymedusan ancestry for the Disconanthae and an Anthomedusan for the Siphonanthae. This diphyletic theory has been generally rejected, with the result that the Disconanths have once more been lumped with the Siphonanth, and their distinctive features unduly minimized or ignored.

These features in brief are, for the Disconanthae, their essentially radial symmetry, with peristomial rings of secondary

polyps acting as blastostyles, their slight degree of polymorphism, lacking nectocalyces, bracts and palpons, their aboral corona of simple tentacles, peripheral canal plexus, and their possession of a highly peculiar central reservoir of nematocysts, penetrated and nourished by an elaborate network of endodermal canals, Haeckel's 'centradenia'.

On the other hand they are linked with the Physophores by their possession of a chitinous float, although this is destitute of a gas-gland and has other peculiarities. Their gonophores have the same Anthomedusan structure, but are ultimately liberated and not permanently sessile or reduced to sporosacs as in most Siphonanthus.

Apart from their centradenia, which is unique in Coelenterates, this combination of characters marks the Disconanthus as much nearer the Hydromedusan type than the highly polymorphic bilateral Siphonanthae, so that in a natural system they should be placed either at the base of the Siphonophora or as an independent branch. Haeckel not unwisely, but on highly speculative grounds, chose the latter course. Whether they can eventually be regarded as primitive Physophorida depends mainly on the homology of the float and on the position assigned to the Calycophora.

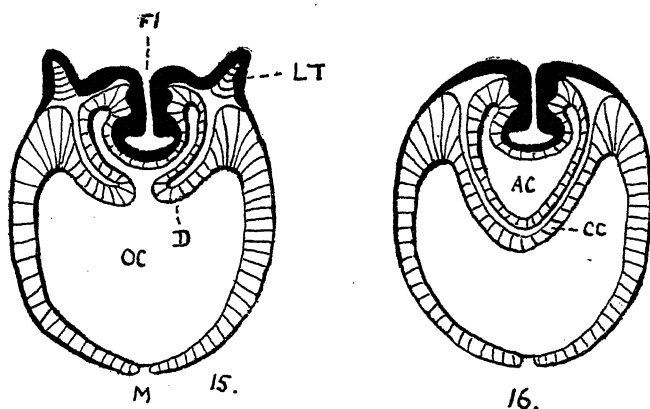
In his opposition to Haeckel's views, Chun (1897 a) took the homology of the float for granted, but, using Chamisso's earlier name, placed the Chondrophora (Disconanthae) at the apex of his system as aberrant Physophores, with the Calycophora at its base. This system is still in general use. He justified this procedure by pointing to Bedot's unfortunate account of a supposed early larva of *Velella* with a single tentacle (1885), which he claimed as evidence of a primary bilateral symmetry of the group, and suggested that the float of Chondrophora might possess a gas-gland in larval deep-sea stages still unknown. The uni-tentaculate larva was subsequently recognized as no larva at all, but a detached 'blastostyle' which had swallowed an air-bubble and carried a gonophore bud as its 'tentacle' (Woltreck, 1904, p. 359). He was equally unfortunate in his suggestion of a larval gas-gland.

Young stages of *Velella* have long been known under the

name *Rataria*, and one of these with eight tentacles and rudimentary sail was carefully described by Chun (l.c., fig. 23). But true larvae were first fished by Woltereck (1904, 1905) in 1903 from the deep water off Villefranche, when the surface was swarming with young *Rataria*. They were found to be not Planulae as in Siphonanthus, but Actinulae already provided with a rudimentary mouth, and in the youngest of all (unfortunately the only specimen) with a pair of short, solid, aboral tentacles, on either side of a narrow, open, involution of the ectoderm, the rudiment of the future float, the orifice of which was temporarily closed by a plug of chitin (Text-figs. 15-16, 21-4). In later stages the thickened lip opens out and develops a projecting circular ridge, which is carried outwards and downwards as the incipient mantle-edge or limbus by eversion and extensive growth of the lip region. Woltereck named this larva *Conaria* from the conspicuous crimson cone of aboral endoderm which projects within the gastric cavity. It is a larval organ of flotation which secretes oil-drops, and disappears by flattening out when the larva reaches the surface. The chitinous plug and fluid contents of the float are then expelled, and the float refills with air. (For further details of the 'cone', see below, p. 129.)

The single aboral pair of tentacles closely resemble those of larval *Narcomedusae* and suggest the possibility of temporary fixation by them in early stages; but they are soon cast off or absorbed, after which the *Conaria* goes through a phase without any tentacles (beautifully figured by Woltereck), which seems to coincide with its period of ascent from deep water, and is followed by the *Rataria* stage, when it reaches the surface and its hollow adult tentacles begin to sprout. These develop by successive diagonal quartets and pairs, at first with strict cruciform symmetry ( $4 \times 2$ ), then with a touch of bilaterality, which Woltereck justly attributed to the influence of the growing obliquity of its float and diagonal sail.

These facts, taken in conjunction with the perfect radial symmetry of young *Porpitas* (i.e. Haeckel's *Discalia* and *Disconalia*), leave no doubt as to the essentially radiate organization of the entire sub-order *Chondrophora*, while the difference in larval type increases the gap between them and the



TEXT-FIGS. 15 AND 16.

Disconantha. Conaria larvae of *Velella* (Diagrams after Woltereck, modified). *AC*, aboral chamber; *CC*, crimson cone; *D*, diaphragm; *FI*, float invagination; *LT*, larval tentacle; *M*, mouth; *OC*, oral chamber.

Fig. 15.—The younger Tentaculate Stage, showing hypothetical origin of the 'crimson cone' as a gastric diaphragm.

Fig. 16.—The later Non-tentaculate Stage, after loss of tentacles and completion of cone.

bilateral Siphonanthae, the larvae of which are invariably solid, mouthless Planulae with unilateral budding.

As regards their float we have to remember that it differs from that of Siphonanthus not merely in the absence of a gas-gland, but in its chambered structure and the peculiar tracheal rootlets, the function and significance of which are still matters of dispute (cf. p. 178, below). Chun's suggestion that a gas-gland might be present in early deepwater stages is now vetoed by Woltereck's discovery that *Conaria* reaches the surface not by the generation of gas, but of oil! For the moment, until some other aspects have been considered, I leave the question of its homology in suspense, the more so as we know at least one other group of Coelenterates, the Minyad Actinians, which have developed an aboral air-float independently. The same may well have happened in these two tribes of Siphonophora.

As the very existence of predators depends on the efficiency of their food-catching apparatus, it is worth noting that the great difference between Disconanthus and Siphonanthus in the form and arrangement of their tentacles appears to be associated with an essential difference in their mode of feeding. That of Siphonanthus is well known: the deadly character of a *Physalia*'s trailing 'long lines' has often been experienced, and the same extensibility, stinging power, and retractility prevail with little change throughout the group. But I have come across no account of the feeding process in any Disconanth, even in the common *Velella*, and in this type particularly the tentacles by themselves seem very ill adapted for the capture of struggling prey. On the other hand, unlike the arrangement in Siphonanthus, the ring of tentacles is closely associated with the peculiar mantle-edge or limbus above it, the margin of which is densely studded with mucus glands (Text-fig. 24, *MG*). Moreover there is a clear correlation between the power of the tentacles and the size (breadth) of the limbus, which is broad in *Velella*, where the tentacles are simple and feebly armed, and narrow in *Porpita*, where they are more or less clavate and studded with nettling knobs. Describing what he regarded as 'respiratory' movements in these forms, Chun writes (1897 a): 'If you observe a living *Velella* or *Porpita*, you will see a peculiar movement in

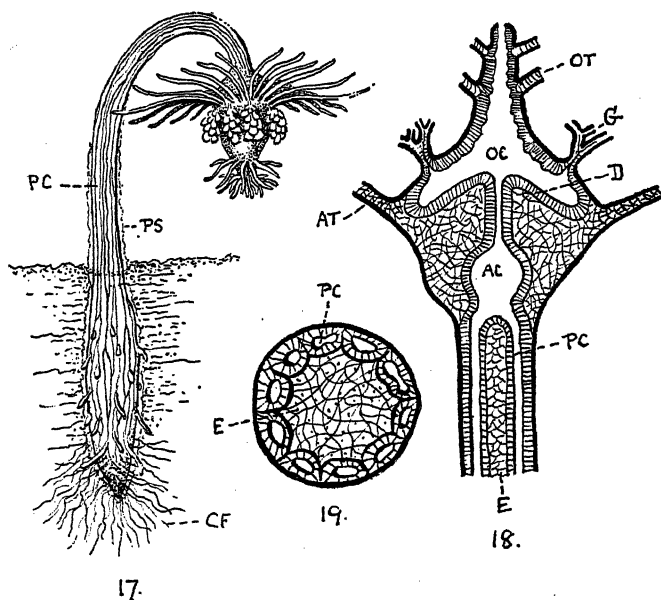
the colony. Twice in a minute all the tentacles bend downwards, while all the feeding polyps contract and the whole under-surface of the body is pressed upwards against the base of the chambered pneumatophore. Then all the appendages slowly resume their resting position.' The respiratory function of the 'tracheae' is still an open question, but, whether these movements are respiratory or not, something more than respiration seems to be involved in them. If the mantle-glands, as seems likely, are engaged in the secretion of mucilaginous threads, the reciprocal up and down movements of mantle-flap and tentacles would afford an excellent means of spreading these threads like a veil over the body as a means of entangling prey.

In any case the structure and armament of Disconanth tentacles are much simpler than in Siphonanth, and almost identical with those of ordinary Hydroid polyps.

#### 4. THE HYDROID RELATIONS OF DISCONANTHAE (Text-figs. 17-19, 20).

Not only are the Disconanths more primitive than the Siphonanth by the preservation of their radial symmetry, the slightness of their polymorphism, and the simplicity of their tentacles, but they show distinct relations with the Tubularian group of Hydroids, especially *Corymorpha*, in their aboral wreath of tentacles, their development of an Actinuloid larva, and the labyrinthine transformation of the aboral region of the coelenteron. With *Corymorpha* their relations are particularly close owing to their production of free Anthomedusan gonophores and the dominance of a large central polyp.

All Disconanth colonies have their zooids radially arranged around a large axial polyp, the coelenteron of which is divided up aborally into a dense network of endodermal tubes (the so-called 'liver'), from the peripheral parts of which the endoderm of the tentacles and 'blastostyles' arises, and from which radial canals ramify over the walls of the float. So also the arenicolous colonies of *Corymorpha* (Text-figs. 17-19) are reduced to a solitary gigantic polyp in which the aboral coelenteron is differentiated into an axial parenchyma (endochord) surrounded by a network of longitudinal branching canals. The gonophores



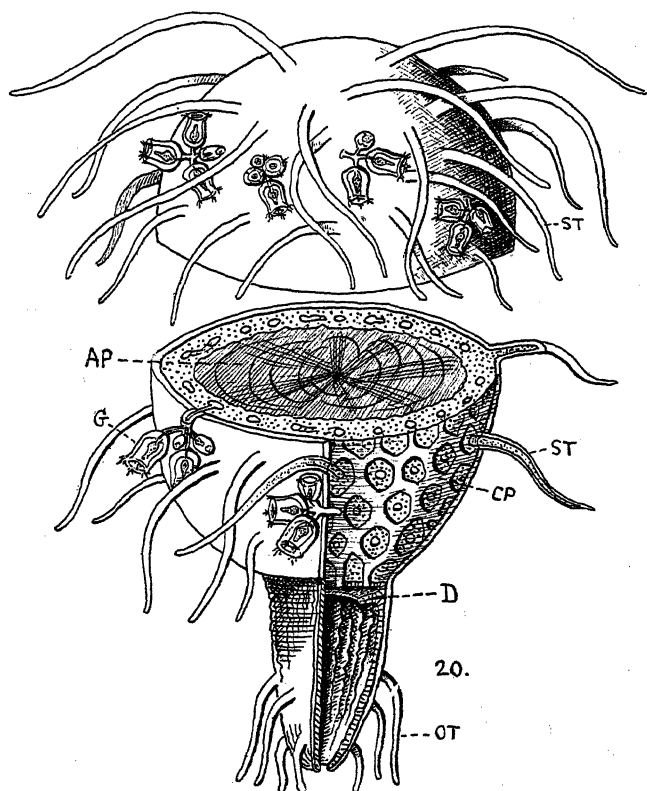
TEXT-FIGS. 17-19.

*Corymorpha*. *AC*, aboral chamber; *AT*, aboral tentacles; *CF*, caudal filaments; *D*, diaphragm; *E*, endochord; *G*, gonophores; *OT*, oral tentacles; *PC*, peripheral canals; *PS*, perisarcular sheath.

Fig. 17.—Generalized diagram of *Corymorpha nutans* and *pendula*, showing habitat and attitude. (Original, after Hincks and Agassiz.)

Fig. 18.—Vertical section of *Corymorpha nutans*, showing gastric diaphragm and openings of stalk-canals into aboral chamber. (Allman, 1871.)

Fig. 19.—Transverse section of stalk. (Allman, l.c.)



TEXT-FIG. 20.

Pelagohydra, showing the stem expanded as a 'float', with axial parenchyma (= endochord) and peripheral canal-plexus, and the hydranth (proboscis) with narrow gastric diaphragm covering the ring of peripheral ostia (original diagram, after Dendy, 1903).

Fig. 20.—AP, axial parenchyma; CP, canal plexus; D, diaphragm; G, gonophores; OT, oral tentacles; ST, swimming tentacles.



arise in tufts from the enteron of the hydranth, as in *Tubularia*.

The ramified endoderm of *Corymorpha* and its allies is limited to the stalk (hydrocaulus). In Dendy's *Pelagohydra* (1903) the sharp distinction between hydranth and stalk at first sight seems to have vanished (Text-fig. 20). In reality the boundary is just as in *Corymorpha*, but the stalk-region or hydrocaulus, with its axial parenchyma and peripheral labyrinth of canals, has been dilated to form a kind of float, and the hydranth with its oral tentacles is relatively reduced. The cauline float is studded all over with tufts of gonophores and long filiform tentacles, the former arising from the endoderm canals, the latter from the parenchyma in their meshes. A special wreath of tentacles on the hydranth in this case is absent.

This remarkable Hydroid is still known only from the single original specimen which was picked up on a New Zealand sand-beach. It was still alive, though moribund, and floated at the surface, mouth downwards, when transferred to sea-water. It even gave faint signs of concerted swimming movements of its tentacles. Its gonophores were fully constituted Anthomedusae with tufts of tentacles at the ends of their four radial canals. Exactly how *Pelagohydra* floats is not yet quite clear, but, from Dendy's account of its histology, it is almost certainly by means of endodermal oil-drops, as in *Conaria*. Further information on its habits and larval form is much to be desired.

In the adult *Velella* or *Porpita* the precise correspondence of the aboral coelenteron with that of *Corymorpha* or *Pelagohydra* is obscured by several secondary changes, partly due to the addition of the centradenial complex, and partly to the development in the stalk of the enormous air-float. The original ring of separate openings to the canals of the peripheral labyrinth becomes lost in the hepatic plexus which penetrates the centradenia; and the originally cylindrical stalk is flattened downwards and expanded outwards, so that the canals which run longitudinally in the stalk of *Corymorpha* now radiate horizontally outwards below the float, and create the illusion of Trachomedusan structure which deceived Haeckel. That this appearance is illusory is clearly seen when we examine

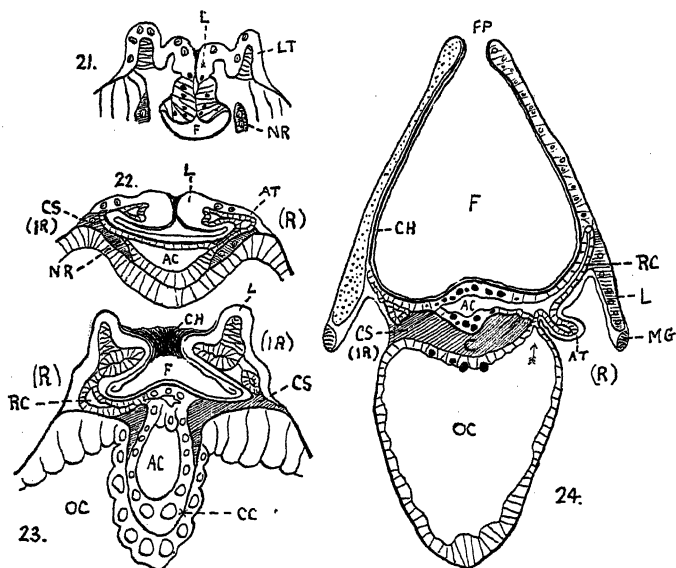
more closely the development of the system in Woltereck's epoch-making larvae.

#### 5. CONARIA AND THE CORYMORPHINES (Text-figs. 21-4).

Like Haeckel's account of Siphonophores in general, Woltereck's terse description of *Conaria* and its development is closely interwoven with a medusoid interpretation of his own, which makes the disentanglement of fact and fancy unusually difficult in parts. His scrupulous workmanship, however, is beyond question, and the features essential to this discussion are borne out by drawings of actual sections which inspire every confidence. The more important of these are here reproduced (Text-figs. 21-4), as well as one of his diagrams very slightly modified (Text-figs. 15-16).

The general features of these early larvae of *Velella* were briefly summarized above (p. 120), and their remarkable internal structure was diagrammatically represented in Text-figs. 15-16. The vacuolated endoderm of the youngest larva, brought up from a depth of some 500 fathoms, was completely destroyed, but has been restored hypothetically from the following stages, actual sections being represented in Text-figs. 21-4. In the earliest stage taken after loss of the larval tentacles the coelenteron is seen to be completely divided into two chambers, a small aboral chamber immediately below the floor of the float, and a large oral chamber, the greatly distended larval stomach. Until the larva reaches the surface as a *Rattaria* this oral region floats uppermost, but I use the terms 'above' and 'below' with reference to the adult orientation, and all the figures are similarly placed.

Wedge between the two layers of endoderm is a flat ring of nematocystic ectoderm, which is seen in the sections to be continuous with the outer ectoderm interradially, i.e. in the intervals between two adjacent radial canals. At their inner ends these radial pieces are united with their neighbours to form a 'nettle-ring', which burrows between the apposed walls of the oral and aboral chambers (Text-figs. 22 and 23) until, in later stages, these walls finally separate from one another (Text-fig. 24), when the ring fills up in the middle to form a solid lenticular



TEXT-FIGS. 21-4.

Conaria and Rataria larvae of *Velella* (vertical sections from Woltereck, 1904). *AC*, aboral chamber; *AT*, adult tentacle; *C*, centradenia; *CC*, crimson cone; *CH*, chitin; *CS*, centradenial spoke; *F*, float; *FP*, float pore; (*IR*), interradian plane; *L*, limbus; *LT*, larval tentacle; *MG*, mucus gland; *NR*, nettle ring; *OC*, oral chamber; (*R*), radial plane; *RC*, radial canal.

Fig. 21.—Aboral region of tentaculate *Conaria*, showing float rudiment and 'nettle-ring' stage of centradenia. Note extreme compression and partial involution of the aboral ectoderm above that of the float.

Fig. 22.—Later stage after loss of tentacles and closure of diaphragm. A radial canal is seen on the right, and an interradian spoke of the nettle-ring on the left (*CS*).

Fig. 23.—*Conaria* with fully developed crimson cone. The aboral ectoderm has opened out, but the aperture of the float remains plugged by chitin. Indications of limbus and adult tentacles.

Fig. 24.—*Rataria* stage. The float is large, chitin-lined, and with apical pore functional. The 'cone' has flattened out; the centradenia is solid; and communications are being formed peripherally between oral and aboral chambers (one marked by arrow).

mass, Haeckel's 'centradenia'. Woltereck attaches a high theoretical importance to the annular character of the earlier stage (see his diagrams in 1904, Text-figs. 15-20), but there is no evidence of a ring-like origin. It probably arises as a series of eight independent interradial proliferations, and the ring-form is merely the first step towards consolidation. Its interradial spokes of contact with the outer ectoderm persist at least to the Rataria stage, when consolidation becomes complete (Text-fig. 24). In the adult *Velella* Bedot (1884) has shown that the organ is separated from the ectoderm by a thin tough mesogloal envelope pierced by a large number of well-defined pores, which permit the passage outwards of cnidoblasts to the appendages. These pores would seem to be the result of extensive subdivision of the original points of ectodermal continuity.

Coming back now to the aboral endoderm, we see the 'cone' in Text-fig. 23 near the height of its development, and projecting into the oral chamber like the manubrium in the bell-cavity of a medusa, though in life the other way up. It is seen to be produced by a downward growth of both endodermal walls, each laden with oil-drops. The pericystic endoderm is already differentiated into the first octoradiate system of radial canals, which are connected distally by a ring-sinus round the lip of the float, and open below into the aboral chamber. There is still no communication between the two chambers. What is the meaning of this extraordinary separation?

Woltereck regarded it as the division between two separate organisms, a primary polyp orally and a medusoid bud aborally, and speaks confidently of the two chambers as the 'polyp gut' and 'medusa gut' respectively. As the 'medusa' has been converted into a pneumatophore it is 'eingesenkt', but the nettle-ring between the two creatures is regarded as a fused vestige of the originally constricted neck of ectoderm—now doubled inwards—between the parent polyp and its medusan bud. Having already remarked on the discontinuity of the connexions between the nettle-ring and the outer ectoderm, I will add only one further comment on this 'Bipolarian' hypothesis. If the centradenial ectoderm really represents the original neck between parent and bud, it is essential that the larval

(‘polyp’) and adult (‘medusan’) tentacles should arise from opposite sides of the dividing nettle-ring. Woltereck completely failed to establish this crucial point. He noted that the inter-radial connexions with the skin (‘Berührungsstellen’) were situated where the thick aboral ectoderm tapers off into the thin pavement epithelium covering the ‘polyp’, and had no difficulty in showing that the adult tentacles arise in the Rataria on the aboral side of this line (Text-figs. 21–4). But of the single tentaculate larva he drew only a perradial section through the tentacles—a radius in which no peripheral spoke of the ring occurs—and says merely that the tentacles lie ‘opposite the ring’, which means nothing (Text-fig. 21). Yet the section clearly shows that the larval tentacles arise from the same thick plate of aboral ectoderm as do the adult tentacles at a later stage, so that, on the evidence submitted, the larval tentacles, like the adult tentacles, belong to the medusa-bud and not to the polyp—which, as Euclid would say, is absurd. There is also no evidence for Woltereck’s view that the adult tentacles arise more aborally than the larval, for the two sets do not occur together, and the mere position of the larval tentacles ‘opposite the ring’ is no criterion.

In fact, as in other cases, the larval tentacles would seem to be simply forerunners of the adult tentacles (e.g. Tubularian *Actinula*), but specialized for some larval function (as in *Myriothele*, p. 146), in this case probably for temporary attachment to the manubrium of the parent gonophore. Such a function would explain the resemblance of these tentacles to those of *Cunina*-larvae, both as regards their restricted number, extreme aboral position, and structure. The peculiar eversion and expansion of the aboral disk after the tentaculate stage implies a previous crowding of these parts aborally, as if to facilitate such a function.

Alternatively let us now turn to some simple facts of Comparative Anatomy. It has long been known that in the Hydroid *Corymorpha* the coelenteron is not merely labyrinthine in the stalk, but is partially divided in the hydranth itself into oral and aboral chambers by a solid annular ingrowth (Text-figs. 17–19), so thick that only a narrow axial passage remains to

connect the two compartments. It is very similar in *Tubularia* (Kükenthal, fig. 386), except that the aboral chamber, following the degeneration of the labyrinth, is itself reduced to a mere vestige (cf. Grönberg, 1898, and below, Text-figs. 50-3). Although the early development of *Conaria* is unknown, it is manifest that a complete septation of the coelenteron which cuts the body off from all connexion with its feeding chamber cannot be primitive either in phylogeny or ontogeny. The septum must have begun as a simple annular fold of the endodermal wall, which, as a larval adaptation, grew inwards and downwards until the central aperture closed altogether, as represented by Woltereck himself in a later diagram (Text-figs. 15-16), freed from the extravagances of his medusoid theory. The condition of an open diaphragm, transitory in *Conaria*, is exactly the condition permanently retained in *Corymorpha*, *Tubularia*, and their monogastric relatives. In *Branchiocerianthus* it is a thin iris diaphragm (Text-figs. 48-9), modified peripherally by adhesions of its upper surface with the peristomial disk. Even in *Pelagohydra* the same division prevails, for Dendy described and figured an annular valve-like fold near the aboral end of the proboscis cavity, which covers the ring of ostia leading to the canals of the labyrinth (Text-fig. 20). In *Tubularia* the longitudinal canals are vestigial (see Grönberg, 1898); but in the others, with the possible exception of *Branchiocerianthus* (in which the exact relations are not yet known), all the longitudinal canals of the stalk open by a peripheral ring of ostia into the aboral chamber defined by the diaphragm.

This arrangement (except for the complete separation of the two chambers) precisely corresponds with the conditions in *Conaria* up to the *Rataria* stage, and confirms the homology of the aboral chambers throughout, for, as shown in Text-figs. 21-4, it is the aboral chamber which receives all the peripheral radial canals that ramify round the walls of the float, the region which obviously corresponds to the hydrocaulus of the Hydroids.

It is worth noting that in the Hydroids mentioned the function of the diaphragm appears mainly to be one of controlling the distribution of food-products from the digestive cavity to

the stalk, whereas that of the crimson cone of *Conaria* is merely one of increasing the oil-producing surface, since the larva takes no external food before the *Rataria* stage when its tentacles grow. It is significant that in *Tubularia* also the gastric epithelium is produced into ridges (the 'pendulous processes' of Allman) which were described by Allman as of a bright vermilion colour from their production of 'clear spherical elements, probably oil-drops', and, with differences of colour, similar phenomena have been described in the adult polyps of various Siphonophora (e.g. *Forskalia*, *Physalia*, *Athorybia*) by Kölliker, Huxley, and Haeckel, usually as *Leberstreifen*.

It remains to add that when the larval *Velella* reaches the surface as a *Rataria*, the cone flattens out, but any reopening of the central aperture is precluded by the ever-growing central-denial ring which forces the two endodermal layers apart and interposes a solid mass between them. But it thins out laterally, and in the radial intervals between its connexions with the skin the two layers meet peripherally and fuse to form a ring of eight ostia, which put the oral chamber into communication with the eight aboral radial canals (Text-fig. 24. Incipient ostium marked by arrow). The ostia subsequently elongate into vertical canals, while the aboral chamber itself breaks up into the 'hepatic plexus' traversing the central-denial mass. These changes explain Haeckel's error in homologizing the adult canal system with that of an octoradiate Medusa. The primary radial canals do not arise, as he thought, from the eight gastric ostia, but from the aboral chamber, and his 'subumbral vessels' are the composite channels brought about by secondary connexions of the primary canals with the oral chamber through these peripheral ostia. The primary radial canals, on the other hand, we have seen to be the strict homologues of the eight or ten longitudinal canals of the stalk of *Corymorpha*, their more regular cruciform or medusan symmetry being the natural result of their development under the uniform conditions of free flotation (cf. Bigelow, 1911, pp. 328, 339, and his fig. B for accounts of the very similar canal systems of Porpitids, and other corrections of Haeckel's descriptions).

Thus both Haeckel's and Woltereck's medusoid theories of Disconanth origin must share the fate of those other medusae which have been invoked to 'explain' the origin of the pneumatophore. The salient features on which they depend are seen to be based on structural conditions already present in various monogastric Hydroids related to *Corymorpha*, so that medusan interpolations are superfluous. *Entia non sunt multiplicanda*.

## 6. THE SIPHONANTH PROBLEM.

The Siphonanthae differ radically from the Disconanthae in almost every respect. They are bilaterally symmetrical; budding is restricted to a single meridian ('ventral'); polymorphism is more pronounced by the addition usually of bracts, palpons, and nectocalyces, or at least of one of these; the gonophores, though Anthomedusan, are with rare exceptions sessile and borne as members of special polymorphic clusters ('cormidia'); there is no general wreath of tentacles, and no central reservoir of cnidoblasts, but each polyp has its own prehensile tentacle and basal store of cnidoblasts ('basigaster'); the coelenteron is simple, though plexiform throughout in one family (Rhodaliidae, Haeckel's 'Auronectae'); the float is simple, but provided with a more or less complex gas-gland: it is absent only in one group (Calycophora), in which, however, nectocalyces are present; lastly the larva, prior to budding, is a solid, mouthless Planula.

These differences present formidable obstacles to the maintenance of Siphonophora as a homogeneous group, especially if Calycophora, as at present, are regarded as primitive. It is noticeable, however, that in many respects the Siphonanth condition is more advanced, less primitive, than that of Disconanths, so that it is worth an effort to see whether the gap between them can be explained as the result of increased divergence of the former from a common source.

In Tunicata which present similar problems in the origin of pelagic from sessile stocks, the original life-history is like that of a Hydromedusan, with the same three stages: a purely locomotive larva, followed by fixation of the larva, and this by an adult stage of feeding and growth, with budding last of all. But in



some forms additional yolk is deposited in the egg, fixation is postponed, and budding is accelerated until it begins in the larva. Finally fixation is abandoned altogether, the original metamorphosis is abbreviated, a nondescript oozoid, neither larva nor adult, but an asexual mixture of both, carries the buds about until they can feed themselves (*Salpa*, *Doliolum*), or a precocious group of buds supersedes it altogether (*Pyrosoma*).

There are grounds for thinking that a sequence of this kind has profoundly affected the character and organization of Siphonanth colonies. For budding begins in the earliest larval stage, the eggs of all Siphonanth are exceptionally large, and in Physophores only one egg is produced at a time—a feature which in Hydroids is associated with the formation of *Actinula* larvae (Lowe, 1926). It seems possible, therefore, that the original larva of Siphonanth ancestors was not a *Planula* but an *Actinula*, and that the Planuloid appearance of Siphonanth larvae is due to the precocity of budding before the typical Actinuloid characters have developed. This view has proved so illuminating that I venture to assume it, and confidently leave it to be judged by results.

It is true that in Calycophora the female gonophore is not so limited in egg-production as it is in Physophorids. From the imperfect data on their ovogenesis at present available I cannot fully explain the contrast, but it is at least possible that the 'polyovon' condition in Calycophora may be consequential on the many modifications (largely simplifications) of structure which the group has undergone during its evolution. In all other Siphonanth the gonophores are produced as buds from specialized secondary polyps (blastostyles or gonopalpons), the real source of their reproductive cells, whereas in Calycophora they arise directly from the budding stem, apparently without the formation of an entocodon (Moser, 1925 *b*), and their ova are endodermal in position from the outset. The single final egg of Physophorids is produced, as in *Tubularia* and *Hydra*, by the fusion or absorption of many young ova: the equally large eggs of Calycophores are predetermined without any such process. Moreover in each cormidium (which will be shown to be the equivalent of a whole Hydroid branch) there is usually only one gonophore, instead of the dense clusters in other Siphono-

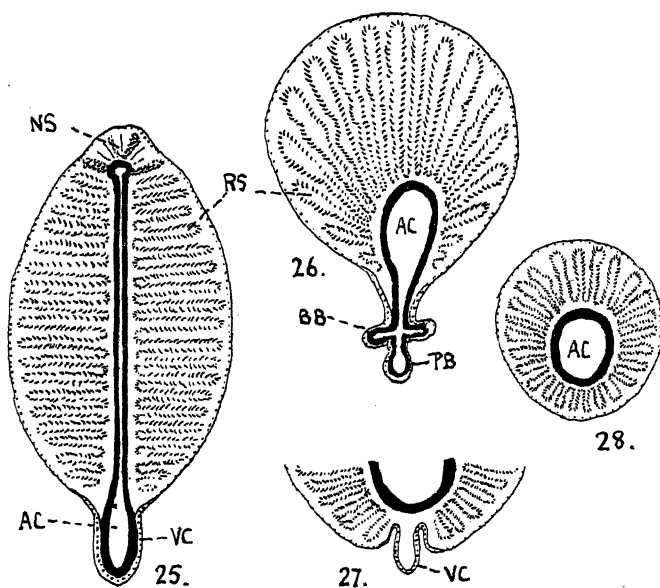
phores. As the whole cormidium is usually detached in Calycophora as a free-swimming reproductive unit ('Eudoxia'), this novel procedure would be futile if each polymorphic cluster were limited to the production of one egg at a time. In any case the subsequent development of the fertilized egg follows exactly the same lines as in Physophorida.

*Dicoryne conybeari* (Text-figs. 34-5) may be an exception to the rule that gonophores producing single eggs give rise to Actinulae, but its eggs barely exceed 0.1 mm. in diameter.

#### 7. GASTRULATION AND THE BUDDING LINE (Text-figs. 25-8).

The eggs of Siphonanthus vary in diameter from something like 0.4 mm. in Calycophores to 0.6 mm. in typical Physonects, a size at least five times greater than that of typical Planula-producing eggs of ordinary Hydroids. They are filled with a highly translucent, peculiarly buoyant sap or yolk. They are liberated as eggs, not larvae, and usually shed the membrane which surrounds them in the gonophore at the time of liberation. They are thus naked from the start, fertilization apparently, and segmentation certainly, taking place outside the gonophore. In some cases (e.g. *Halistemma rubrum*) the eggs are so large that the segmentation stages can be followed without the aid of a lens (Metschnikoff, 1874).

It follows that no distinction can be drawn between embryo and larva, except by analogy. The larger and yolkier larvae preserve the spherical form of the egg long after the first larval organs and buds have arisen (e.g. *Agalma*, *Crystallodes*, *Athorybia*), and these in all cases make their appearance long before the endoderm has completed its delamination from the yolky cells of the primary morula. The germ-layers are in fact first formed along a particular meridian, and then extend to right and left, first the ectoderm, then the endoderm more slowly until gradually the whole surface of the yolk is covered. There are variations in detail, and there is a tendency towards acceleration of the process aborally, but the region opposite the meridian of first formation is the last to be differentiated. The primary or zero meridian is naturally also the site of precocious



TEXT-FIGS. 25-8.

Stems of *Macrostele Siphonanthus*. Diagrams of transverse sections.  
*AC*, axial canal; *BB*, bract buds; *NS*, nervous system; *PB*, polyp bud; *RS*, radial septa of mesogloea supporting muscle fibres; *VC*, ventral crest.

Fig. 25.—*Forskalia ophiura*. (After Korotneff, 1884.)

Fig. 26.—‘*Agalmopsis*’ *sarsii*. (After Claus, 1878, modified.)

Fig. 27.—*Apolemia uvaria*, internode. (Korotneff, l.c.)

Fig. 28.—*Praya diphyes*, internode. (Korotneff, l.c.) Calycophorida.

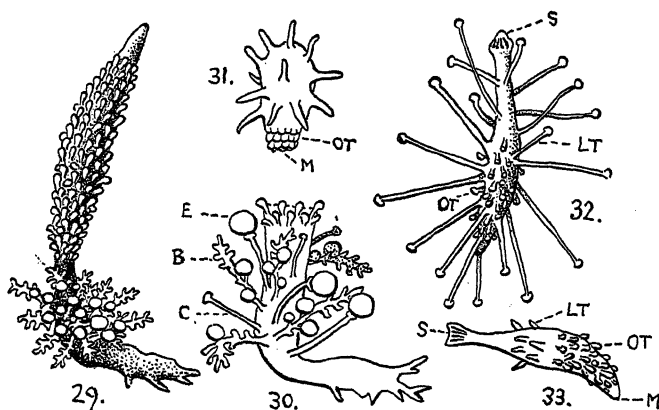
Physophorida

budding, and was conveniently termed 'ventral' by Claus and Haeckel. The term, of course, has no necessary relation to the orientation of the animal in space (pace Schneider, who objected that a *Sphaeronectes*, when swimming, carries its budding line uppermost).

There can thus be no doubt as to the precocity of budding. Buds arise in the first patch of the embryo that has acquired its diploblastic coat, while the rest of the body is solid with yolk—long before there is any possibility of it showing typical Actinuloid features. It would thus appear that the slow bilateral course of gastrulation, combined with the precocity of budding, is the prime cause of the unilateral 'budding line' and the bilateral symmetry of Siphonanth colonies.

This initial factor is reinforced, as development proceeds, by histological differentiation of the larval body-wall, which, outside the ventral tract, becomes increasingly thickened and specialized by the development of a powerful musculature and radial sheets of mesogloea to support it. A cross-section of the stem of any long-stemmed Siphonanth reveals a narrow central lumen and, between ectoderm and endoderm, a thick ring of longitudinal muscle-fibres (ectodermal) arranged in close-set radial series on each side of corresponding centrifugal bands of mesogloea (Text-figs. 25-8). This elaborate muscular envelope tapers from the dorsum down each side to the ventral line, where it is replaced by a thin fold of the undifferentiated primary layers, the so-called 'ventral crest'. Only along this tract is budding possible, and there are limitations even here. In *Forskalia* and the Agalmids the crest is continuous, thus permitting the formation of 'dissolved cormidia' below and tiers of nectocalyces above; but in forms with 'ordinate cormidia' the crest is interrupted along the internodes of the stem, either by lack of the endodermal element (e.g. *Apolemia*) or by complete suppression of the crest (*Calycophora*) outside the segmental nodes.

Korotneff (1884) to whom, after Claus (1878), this survey is mainly due, arranged these modifications in the reverse order, which he thought represented successive grades of departure from Hydromedusan conditions, as presented by the 'solitary'



TEXT-FIGS. 29-33.

*Myriothele*. Life-history of British species. *B*, blastostyles, coryniform, bearing gonophores below; *C*, 'clasper' (modified tentacle?) which withdraws a ripe egg from gonophore; *E*, egg (or embryo) held by clasper; *LT*, larval tentacles; *M*, mouth; *OT*, oral tentacle; *S*, sucker.

Fig. 29.—Colony with blastostyles. (Hinks, 1868.)

Fig. 30.—Blastostyle region enlarged. (After Allman, 1875, simplified.)

Fig. 31.—Larva on hatching, oral end below.

Fig. 32.—Larva fully formed, with larval tentacles extended and proboscis developing; sucker (*S*) above.

Fig. 33.—Larva on fixation, larval tentacles withdrawn. (Figs. 31-3, Allman, l.c.)

Gymnoblast Myriothela. But those conditions are much more closely approached in the body-wall of Physophora (Korotneff, l.c.) and doubtless of other 'Brachysteles' (though in none of these are figures of the ventral line available), as well as in the long-stemmed Cystonects Rhizophysa and Bathypysa. In any case it seems clear that in Siphonanth's generally the post-larval structure of the stem (i.e. of the body-wall of the oozoid which bears the colonies) prohibits all budding except along the ventral tract, and there only in a limited belt at the base of the protosiphon.

Other relations of the ventral tract to processes of growth will be dealt with in the sequel.

#### 8. THE NATURE AND ORIGIN OF BRACTS (HYDROPHYLLIA) (Text-figs. 29-33).

These structures in their manifold variety are perhaps the most characteristic of Siphonanth appendages, but their history, in the perpetual wrangle over polypoid and medusoid theories, has never been made clear. To Haeckel they were in some cases the split and isolated umbrellas of ancestral medusae, and in others simply elongated and degraded nectocalyces—the former view being based on his early observation of larval Physophora, the latter on his alleged detection of vestigial nectosacs at the tips of the coronal bracts of several forms, notably 'Athoria' and 'Rhodophysa' (1888). Unfortunately the single specimens of these forms, which had been seen by Haeckel only, have been lost, and his idealized pictures of the former (Pl. xxi, 5 and 6) can scarcely be regarded as evidence; for the tip of a larval bract is so frequently provided with a nest of large nematocysts, sometimes four in number, set up side by side in a little terminal pocket, that a lively imagination could easily convert this into a minute vesicle segmented by four little radial canals (cf. Haeckel's Pl. xxi, figs. 10 and 12, with Metschnikoff's figures of larval Agalma, 1874, or Schneider's special drawings of them, 1896, Pl. xliii, 1-3). Moreover it should be noted that in his original description Haeckel's words were less emphatic than his figures, for all he said was that 'four very small radial canals seem to arise from it (i.e. the bracteal canal)' (l.c.,

p. 202). If to Haeckel's keen eye they only 'seemed', we may certainly discount the hard strokes of his pencil, as well as his subsequent overstatement that these 'seeming' nectosacs are a 'proof that the bracts in these cases are reduced nectophores'.

Oddly enough Schneider claimed these same structures in larval bracts as proofs of their polyp nature. Like Claus he took the terminal pockets to be the mouth-openings of bracteal canals, which became shut off from them in adult *Agalmas*, but remained open through life in the 'very primitive' *Calycophore Praya*. Such openings were later denied by Moser from her own observations at Naples (1924, p. 27).

Bracts as swimming organs were tersely described by Huxley in his account of *Athorybia* (1859, p. 88, Pl. ix), but more fully by Kölliker (1853, pp. 24-5), whose account I translate: 'Swimming bells are altogether absent, and replaced by a crown of solid covering-bracts which, from their attachment by moveable stalks, serve not only for protection of the remaining parts but also for swimming. . . . Both singly and all together they make energetic movements, which appear as a rising and falling of the individual leaflets, and, when all work together, they bring about a self-opening and self-closing of the composite crown. When an *Athorybia* rests at the surface of the water, quietly lurking for prey, often all its bracts are widely unfolded and it has the form of a perfectly flat shell [admirably represented by Haeckel, Pl. xi]; but if one touches it, however gently, it instantaneously shuts up, so that the points of all the bracts meet below, the animal assumes an oval shape, and the polyps and other organs are completely enclosed (Pl. vii, 1). Still more striking are these movements when an *Athorybia* swims, always by the activity of its bracts alone, which, without any alteration in their shape, but opening and closing alternately as a whole like the umbrella of a *Medusa*, drive it forward in the direction of its pneumatophore, through the combined impulse of the water contained in the common cavity.'

Thus the coronal bracts subserve passive flotation or active locomotion as required, and provide a protective cover for the retracted tentacles. Of these functions that of locomotion would

seem to be the chief, since in larval Agalmids (which closely resemble the adult *Athorybia*) all the bracts are shed as soon as nectocalyces become functional. Confirmation of this view is provided by *Stephanomia* (*Halistemma*) *rubrum*, the largest of Mediterranean Agalmids, in which the nectocalyces make their appearance exceptionally early, and the larval bract either fails to appear (Metschnikoff, 1874) or has a very short and precarious existence (Woltereck, 1905 b).

The early development of *Athorybia* was traced by Haeckel (1869, Pl. xiv) and is practically identical with that of *Agalma* and *Crystallodes*. The first appendage is a smooth gelatinous subapical bract, which extends backwards over the antero-ventral budding area of the Planula, as in *Physophora*. It arises extremely early, even before the float, its axial canal springing from the earliest patch of gastral cavity. Then the typical leaf-shaped bracts arise beneath it and radiate outwards from their ventral attachments so as to enclose the whole embryo. The pneumatophore at length pushes up between them, the primary bract is thrown off, and the secondaries continue to multiply on both sides to form a bilateral corona, as in *Anthophysa* (Text-figs. 45-7).

At this stage in Agalmid larvae the first nectocalyces arise on the stalk of the pneumatophore, and the larval bracts are soon afterwards thrown off. But they persist and continue to multiply, as we have seen, in *Athorybia*, although rudimentary 'nectocalyces' may arise in front of them which never function (Chun, 1897 b), and in *Nectalia* they persist and become remarkably differentiated (see p. 172) along with functional nectocalyces and a float. As these are short-bodied forms, it would seem that the disappearance of the coronal bracts in long-bodied forms like *Agalma* is partly due to the elongation of the stem, which develops its own bracts, often in great profusion (cf. p. 153, below).

These stem-bracts in the long-bodied *Physonects* are present in hundreds, even thousands, and would seem to play an important part in the adaptation of *Macrostele Siphonanth*s to a life of incessant vertical migrations. As the Copepods, Euphausiids, and other prey periodically rise and sink, many *Siphono-*



phora must do the same or starve; so during their slow descents—it may be through several hundred fathoms—the bracts will probably all be spread out horizontally, like the coronal bracts of *Athorybia* when quietly drifting, and during the quicker ascents, whether by nectocalyces or pneumatophore, will be pressed against the stem to reduce resistance. In a few forms they are transformed into cubical or prismatic gelatinous floats, which may be found with more knowledge to be indicative of a more restricted range of depth. The complete absence of bracts as well as nectocalyces in *Cystonectae* is doubtless to be correlated with the great size and perfection of their float, in which gas-pressure can be adjusted by its aperture, and thus control both ascent and descent. *Anthophysa*, as we have seen before, is again the intermediate link, since, along with a large but closed float, it retains paddling bracts, but lacks nectocalyces.

In *Calycophorida* only the stem-bracts survive as free structures, one in front of each cormidium, which they serve partly as a protective cover, and partly, in the case of free *Eudoxids*, as floats. They are highly gelatinized, and in the latter case assume fantastic shapes, prismatic, cuboidal, polyhedral, but in their simplest form they resemble the primary larval bracts of *Physonects*, pointed distally, convex externally, and concave towards the cormidium, which they more or less embrace. In *Eudoxids* their axial canal is usually much dilated as the so-called 'phyllocyst', the walls of which are highly vacuolated, and usually secrete a terminal oil-drop as a float. Only in *Polyphyidae* are bracts entirely lacking, the cormidia being sessile and the whole stem retractile within the cavity of the strangely built spire of nectocalyces. (*Hippopodius*, p. 157).

But, although bracts are not present in the nectosome of *Calycophores* as independent organs, it was pointed out by Schneider (1896) that the more gelatinous of their nectocalyces, especially the primary bells, by the possession of a *Sapfbehälter* or somatocyst—the exact equivalent of a phyllocyst—betray themselves as compound structures formed by fusion of bract and bell, which he distinguished accordingly as *Deckglocken* (i.e. bract-bells) in contrast to simple *Schwimmglocken* or nectocalyces. As can be seen in *Metschnikoff's*

figures of *Galeolaria* ('*Epibulia*') or Chun's of *Mugiliaea* (Text-figs. 2 and 3), the somatocyst arises from the small gastral cavity of the young *Planula* close to the base of the larval nectocalyx, between the latter and the apical pole, exactly where the cap-shaped bract of a larval *Physophore* takes its origin. On the strength of these relations Schneider—as I think on ample grounds—homologized the endodermal canals of the two structures, thereby establishing an important common feature of *Calycophore* and *Physophore* larvae. The identity of the two structures was subsequently recognized by Woltereck (1905) and Moser (1924, p. 25), and used to support their argument that the larval bract, and not the pneumatophore, of *Physophores* corresponds to the larval bell of *Calycophores*.

Unfortunately Schneider shared with the last-named authors the same fixed idea as to the primitiveness of *Calycophores*, and all three missed the real significance of his identification. The simple inference was, of course, that the independent primary bract of the *Physophore*, already verging on disappearance, had become secondarily fused with the nectocalyx in the *Calycophore*. Instead he tortuously interpreted the independence of the bract in *Physophores* as due to a secondary breakdown of the *Calycophore* 'bract-bell', by which the bell became the pneumatophore, and the somatocyst the bract! He then proceeded to re-classify the *Calycophora* according to the dominance or degeneration of the bract-bell, broke up Chun's nicely ordered sequence of Mono-, Di-, and Polyphyids, based on the number of their bells, and drew the great man's thunders on his head as the author of '*kein System der Siphonophoren, sondern die Caricatur eines Systems*' (1898 a, p. 305). Nevertheless, although Schneider's systematic adventure was ill-conceived, Chun never adequately met the morphological case which prompted it, as Schneider bitterly complained (1898), and I revive it now in the full belief that it is unassailable. I interpret it as showing that, in the earliest days of their history, *Calycophores* as well as *Physophores* possessed larval locomotive bracts, probably before nectocalyces had come into existence. With their increasing precocity the first or larval bract was transformed into a transitory gelatinous shield over the budding

area, and the locomotive function in Physophores was handed over to its successors, while in Calycophores the precocity of the primary nectocalyx rendered all locomotive bracts superfluous, and the primary bract was incorporated in the nectocalyx, which took over the protective function itself (cf. the hydroecium in Text-figs. 5 and 12). The bract being subapical, and anterior to the nectocalyx, the effect of their fusion was to provide the nectocalyx with an anterior pyramid of jelly as the functional apex of the young colony, in place of the larval apex which, as we saw earlier, had meantime been absorbed.

As evidence of the original independence of the somatocyst-bract, I have reproduced another neglected figure, also of Metschnikoff's (1874, Pl. vii, 16), which represents a larval Calycophore, fished at Villefranche, and provisionally identified as *Praya inermis* (?) (Text-fig. 6). The gelatinous dome over the somatocyst is seen to be incompletely fused with the nectocalyx. Bigelow regards Metschnikoff's species as *Sphaeronectes truncata*. There seems to be no ground for Moser's suggestion that this larva may be a detached cormidium (1924, p. 16).

If my assumption is correct that the original larval form of Siphonanthus was an Actinula, as it still is in Disconanthus, these bracts assume a new significance. They are neither modified polyps nor modified medusoids, but represent the persistent tentacles of the original Actinula larvae more fully adapted to the locomotive and other functions which we have seen they discharge in the young pelagic colonies.

Now the Actinula is merely a Planula with precocious adult characters, or, to be more precise, a Planula provided with additional yolk so that it develops some at least of the future polyp tentacles as well as a mouth before its liberation. Apart from the little four-tentacled larva of *Coryne Van Benedeni*, we are acquainted with only two types of Hydroid Actinula, the Tubularian (or Corymorphine), with an equatorial ring of tentacles (i.e. the future 'aboral ring' of the Hydranth with a considerable rudiment of the stalk), and the Myriotheline, with long tentacles scattered all over its body (Text-figs. 29-33). In both cases the tentacles are locomotive, mainly ambulatory

perhaps, but in *Tubularia* at any rate capable of swimming. The larva of *Myriothele* has been little studied since Allman (1875) described its remarkable history. I give here a short account of this history, for in various respects in which *Siphonanthus* differ from *Chondrophora*, *Myriothele* shows unexpected points of agreement. [See Postscript, p. 193.]

*Myriothele* is another of the solitary or monogastric *Gymnoblasts*, but unlike *Corymorpha* and its allies, it is firmly attached by one side of its short stalk to rocks or large stones. Its body is very extensible, and closely studded with short knobbed tentacles, but, unlike any other Hydroid, it lies more or less prone on the face of a rock, or on the underside of a flat stone, and there stretches and probes about with its long proboscis for passing prey. It has peculiarly branched *Coryniform* blastostyles behind, presumably the vestiges of a once fully colonial life. They bear adelo-codonic gonophores, each of which, when female, liberates a single large egg, the fertilization and development of which take place outside the gonophore. But, instead of being sent adrift, as in *Siphonanthus*, the egg, still enclosed in its membrane, is seized by a specialized tentacle (or *dactylozoid*) near by, and held aloft during the whole of its subsequent development. In this position the embryo from the beginning develops freely outside the gonophore on its own resources, and to that extent resembles the egg of every *Siphonanth*, and differs from those of *Chondrophora* and the *Tubularian* Hydroids (including *Corymorpha*), which are nursed within the parent gonophore until the *Actinuloid* characters are complete.

In the first years of the Plymouth Laboratory the late Sir William Hardy, then a young man fresh from Cambridge, paid several visits with the object of working out the development of *Myriothele*, and I have pleasant recollections of helping him in the search for specimens. Unfortunately he gave up this intention on learning that Korotneff was engaged on the same problem, a task he never completed. Nevertheless Hardy made the discovery (1898, fig. 13) that *Myriothele* is not limited to the budding of blastostyles, but produces little clusters of polyp-buds in the region immediately behind the blastostyles, though

they develop by a peculiar method and break off as in *Hydra* and *Tiarella* (Text-figs. 54-7). [See Postscript, p. 193.]

The larva before hatching has an oval body bearing short blunt tentacles except at the narrow oral end, which is closely beset with several rows of tubercles, forerunners of the adult series of short capitate tentacles (Text-fig. 31). This end grows rapidly, making the body spindle-shaped after hatching (Text-fig. 32). The larval tentacles become elongated and definitely capitate, while additional ones arise in the oral extension among the short adult tentacles, possibly by the enlargement of some of them. The aboral extremity develops a terminal sucker, which is apparently invaginated. After a free life of some days the larva attaches itself by its sucker,<sup>1</sup> and the long larval tentacles are withdrawn (Text-fig. 33). A remarkable developmental feature is the precocious origin of the hollow larval tentacles in an involution condition (Allman, 1875). [See Postscript, p. 193.]

It would seem, therefore, that in *Myriothela* there is only one diffuse set of tentacles, as in *Coryne*, of which the larval are merely an aboral selection precociously developed for larval purposes. There is thus a close correspondence between the larval tentacles of *Myriothela* and the larval and paddling bracts of *Siphonanthus* both in their aboral position, locomotive function, and precocity.

The sharply defined circlets of the Tubularian *Actinula* are much less closely comparable, although there also the aboral tentacles develop before the oral. When the larva of *Pelagohydra* has been found, it may well provide a bridge between these two extremes. *Conaria*, with its vestigial pair of tentacles and internal complexity, is clearly too specialized for comparison (cf. p. 120).

#### 9. NECTOSOME AND SIPHOSOME (Text-figs. 34-5).

Haeckel's distinction between the locomotive fore-body (nectosome) and the nutritive and reproductive hind-body (siphosome) is well based. The former carries the float, necto-

<sup>1</sup> So Allman states (1875); but his figure (my Text-fig. 33) is not convincing, and Sars is emphatic that in *Myriothela arctica* the larva attaches itself by several posterior tentacles, and not by its sucker.

calyces and paddling bracts, the latter the polyps and gonopalmes (= blastostyles), as well as various sterile palps and stem-bracts when present. Each zone has its own centre of proliferation in the ventral tract, the two being separated by a slight gap.

In the larval stage the two zones correspond essentially with the hydrocaulus and hydranth of the primary polyp of a Hydroid colony, but the divergent modes of budding and the varying relations of gonophores to stem and hydranth prohibit any rigid homology. Nevertheless it is remarkable that the cauline 'float' of *Pelagohydra* is exactly equivalent to the nectosome, and carries an equipment of locomotive tentacles and gonophores, the theoretical predecessors of paddling bracts and nectocalyces. These tentacles, so alien to the stalk of an ordinary fixed Hydroid, will doubtless be shown in time to be the persistent tentacles of an Actinuloid larva (cf. *Myriothela*, p. 146). Their diffuseness, and the presence of gonophores among them—both cauline—also point away from the Corymorphine type towards *Myriothela* and the Corynoids.

(a) Nectosome.—Although paddling bracts and nectocalyces are both characteristic of the nectosome, it is important to note that they are alternative organs and rarely (*Nectalia*) function together. In the development of Agalmids the bracts come first and act as larval organs of locomotion, but they atrophy when the nectocalyces develop—a fact which strongly suggests that bracts were the earlier locomotive organs and are here in course of replacement. In *Nectalia* they are specialized for steering, and have ceased to paddle (p. 175). When nectocalyces are especially precocious, as in *Halistemma rubrum*, the larval bracts disappear very quickly or even fail altogether (cf. p. 141). This precocity of replacement reaches its climax in *Calycophora* (cf. p. 143).

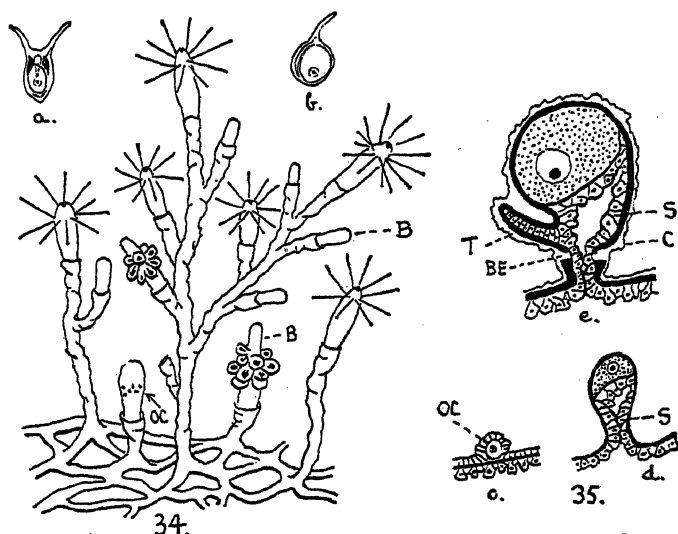
On the other hand paddling bracts persist throughout life in *Athorybia* and *Anthophysa*, and in the former Chun (1897 b) discovered rudimentary medusoid buds in front of them. These have been generally regarded as vestigial nectocalyces, but, with *Pelagohydra* in mind, one may tentatively claim them as obsolescent Hydroid gonophores, which in other families

have been converted into nectocalyces. Chun traced their development, but thought description unnecessary, and indicated no distinctive features. They urgently need a closer examination from this standpoint. In *Anthophysa* they have disappeared altogether.

(b) *Siphosome*.—When the larva of an arborescent Hydroid has fixed itself and grown into the primary polyp or oozoid, its budding zone for new polyps lies below the hydranth proper, between it and its stalk of fixation, exactly as the budding zone of a *Siphonanth* lies between the primary 'polypite' and its locomotive nectosome. From this zone in the growing Hydroid new polyps arise as radial or lateral buds, usually all alike and fertile, producing their own gonophores (e.g. *Syn-coryne*), but occasionally dimorphic, the terminal polyps of branches remaining permanently as sterile gastrozooids, the secondary subterminal polyps being specialized from the outset as mouthless blastostyles or 'gonozooids' (e.g. *Dicoryne*, Text-figs. 34–5). The latter is the condition in all *Siphonanth*s, with the possible exception of the *Calycophora*, in which Chun's identification of a blastostyle is disputed (Schneider, 1898, 116; Moser, 1925; versus Chun, 1897 a). With the doubtful reservation of that sub-order, each cormidial group consists in the simplest cases merely of a gastrozoid (the siphon or polyp) and a gonozoid (the gonopalpon and its gonophores), though complicated in many cases by the addition of bracts or palpons or both.

Thus, in spite of the rarity of blastostyles in *Gymnoblast*s, there is no sharp difference between *Siphonanth*s and *Hydroids* as regards their polymorphism. In their essential constitution the cormidia of *Siphonanth*s, morphologically and physiologically, represent the trimorphic reproductive branches of an arborescent colony of the *Dicoryne*<sup>1</sup> type, in each of which a mouthless blastostyle and its gonophores are nourished by a

<sup>1</sup> In spite of its name *Dicoryne*—like *Podocoryne*—is not a *Corynid* in the systematic sense, but belongs to the *Hydractinia*—*Eudendrium*—*Bougainvillea* group, with a single whorl of tentacles above the gonophores and a bare hypostome. The only *Corynid* with blastostyles in these waters is *Tubiclava* and that is not arborescent.



TEXT-FIGS. 34 AND 35.

*Dicoryne*, its blastostyles and locomotive sporosacs. *B*, blastostyle; *BE*, breach in ectoderm; *C*, cuticle; *E*, egg; *OC*, oocytes in blastostyle; *S*, spadix; *T*, tentacle, with chordoid axis.

Fig. 34.—Generalized diagram of a typical colony, based on *Dicoryne conybeari* (combined from Allman, 1871, and Ashworth and Ritchie, 1915). In *Dicoryne conferta* the blastostyles have pointed tips, but polyps and branching are much the same in both species. Above, two ciliated sporosacs: (a) *Dicoryne conferta*, bi-tentaculate, usually containing two eggs, (b) *Dicoryne conybeari*, uni-tentaculate, usually with one egg.

Fig. 35.—Development of sporosac on blastostyle of *Dicoryne conybeari* (Ashworth and Ritchie, l.c.): (c) oocyte in ectoderm, (d) outgrowth of spadix, (e) ripe sporosac with tentacle, ready for liberation.



terminal polyp. The fact that in most groups the cormidia include additional elements, such as bracts and palpons, in no way weakens the comparison, for, in contrast to Hydroids, Siphonophores have had to face a far greater variety of novel conditions of life, and to meet them by corresponding modifications of form and structure. Primary and secondary functions of the bracts have been discussed above. Palpons are no less variable in type, and doubtless play different parts in different families. They may serve as tactile, and even as prehensile organs in *Physophora* and *Epibulia*, as supplementary organs for circulating the food-stream in others, or as ampullae for storage of fluid during sudden contractions of long stems. These are all special problems awaiting settlement by observation, and in creatures far more difficult to capture without injury and to observe than Hydroids.

The outstanding difference between the reproductive branches of Hydroids and Siphonanthus is in their arrangement—a perpetual forking in Hydroids, a linear succession in Macrostelgia, and a cyclical multiplication in Brachystelia. We are now in a position to give consideration to these points.

#### 10. CORMIDIAL BUDDING IN MACROSTELIA.

Hitherto the uniserial, metamerised stem of the Calycophora has been regarded as primitive (Haeckel, pp. 7–9), and the derivative nature of the other types has been taken for granted. Phylogenetic value has even been given to the frequent detachment of the cormidial segments in Calycophora as free-swimming ‘Eudoxiae’. Schneider (1896) saw in this phenomenon a recapitulation of the process by which free-swimming Siphonophores first arose from highly polymorphic sessile ancestors, while Moser (1925) regarded it as older still, a step towards the evolution of ‘true Medusae’ out of her fantastic ‘Urmedusen’ (cf. p. 117, *supra*).

It is not irrelevant to note the steps by which Schneider reached his strange conclusion:

- (i) The pneumatophore arose as a modification of the larval nectocalyx of Calycophora (l.c., p. 577);

- (ii) Therefore all Physophores, including Cystonects and Chondrophores, were derived from Calycophora (p. 611);
- (iii) Phylogenetically, therefore, only the 'simply built ancestral Calycophora' need be considered ('Die übrigen sind, weil von den Calycophoren ableitbar, auszuschalten', p. 632);
- (iv) Within the Calycophora the most ancient types alone exhibit the detachment of polymorphic cormidia as Eudoxiae (p. 653);
- (v) All other Siphonophores have 'lost' this habit (ibid.);
- (vi) Therefore the detachment of cormidia is not a newly acquired feature, but was inherited from polyp-stock ancestors (ibid.)!

Needless to say the whole argument is invalidated by its basal assumption, and by the fact that the Physophorid groups which were excluded from consideration contain the very forms whose cormidia are simplest in constitution and exhibit a polymorphism no greater than that of many existing Hydroid stocks. Schneider made no attempt to show how the independent lateral branches of the ancestral Hydroid came to be transformed into the single metameric series of the modern Calycophore. Let us now examine this problem.

Each tetramorphic cormidium of *Sphaeronectes*, according to Chun (1897, my Text-figs. 12-14), makes its first appearance on the stem as a single pro-bud, which subsequently divides up into its four components—bract, gonophore, tentacle, and polyp (provisionally I follow the traditional treatment of the 'tentacle' as a dactylozoid). But, as soon as the first pro-bud has arisen, it is carried backwards by elongation of the stem, and a second pro-bud arises in front of it. The same process is repeated indefinitely, and each pro-bud in turn begins to resolve itself into its heterogeneous components. A continuous succession is thus kept up, and the stages of differentiation can be followed by glancing along the series from the youngest pro-bud to the fully constituted polymorphic groups at the end of the chain (Text-figs. 12-14). The succession of developing cormidia thus resembles the stream of proglottides from a *Taenia* scolex, or the chain of buds in a *Salpa* stolon, but with this

difference, that in these cases the buds, as they leave the stolon, are all alike and share the same fate, whereas in the Calycophore each soon begins to sprout into its heterogeneous elements, first polyp and tentacle distally, then gonophore and bract at its base. It is in fact the most condensed and complicated type of budding in the animal kingdom, and implies the existence of an internal machinery of differentiation without parallel in other cases. Even in *Doliolum* the pro-buds, as they leave the stolon, appear to be all identical, and their ultimate differentiation seems to be dependent on the positions to which they are carried by their attendant phorocytes. There is no possibility of epigenesis in the formation of the cormidial buds of Calycophores.

It is true that Chun's account was disputed by Schneider, who claimed that the gonophore-bract bud arises independently of the polyp bud, but his figures of *Abyla* (1896, figs. 15 and 18) are by no means decisive, and in any case the duplicity of the gonophore bud requires explanation. To Schneider himself it was just another form of his 'bract-bell' (cf. p. 142), which in the nectosome he thought was eventually dismembered to form the pneumatophore and larval bract of Physophorida; but I doubt if any one else could imagine that a bract-bell, once consolidated, could freely resolve itself into its original components. Moreover the bract-bell is a sterile nectocalyx, and, however easy the transformation may be of a gonophore into a nectocalyx, there are serious obstacles against the conversion of nectocalyces into gonophores.

Nothing like this condensation of the budding process occurs in any Physophorid. With the exception of the siphon-tentacle pair, all the buds arise from separate rudiments wherever their origin can be followed. When the cormidial tufts are restricted to definite 'nodes' along an elongated stem, they are usually too crowded for exact determination, but in *Cystonects* which lack bracts and palpons, as well as in various 'Brachysteles', the primary independence of trophozooids and gonozooids is easily verified (cf. Haeckel, Pl. xxiv).

Moreover outside the Calycophora the restriction of buds to a single longitudinal series is not absolute. In *Forskalia* each

polyp is embraced at its base by a pair of bracts, which implies the existence of a triserial budding tract with lateral as well as median buds. The same is true of various short-stemmed Agalmids, the cormidia of which have been carefully described and figured by Bigelow (1911). In *Agalma okeni* (= Haeckel's *Crystallodes*) and *Stephanomia amphitridis* there are three or four pairs of bracts in each cormidium springing from the stem on either side of a median series of siphons, palpons, and gonozooids (cf. Text-fig. 26). In *okeni* the bases of the bracts in any one cormidium lie in the same transverse plane (i.e., xvii, 9 and 13); in *amphitridis* in a pair of oblique rows, the most dorsal bract being distal, the most proximal ventral (xviii, 2). This arrangement is explained by a section of Bedot's (1888, Pl. iv, 28) through the stem of *Agalma clausi*, in which the ventral half of the rather stout triangular stem is much less muscular than usual. The pedicels of the bracts are seen to arise from the sides of the non-muscular ventral half at various distances from the ventral edge, the most dorsal lying just within the muscular zone. They are clearly lateral buds, and appear to have been formed successively in pairs near the ventral edge, and then pushed dorsally in turn by successive increments of transverse growth. The obliquity of the rows in *amphitridis* would follow from a larger element of longitudinal growth.

The remarkable carapace which surrounds the siphosome of *Agalma 'eschscholtzii'* (= *Agalma haeckeli*, Bigelow) was described and beautifully figured by Haeckel (xviii, 8) as consisting of bracts arranged in a 'continuous spiral'. In view of the admittedly close relationship of this to the foregoing group of species it is highly probable that its bracts were attached to the stem by similar segmental groups of pedicels with bilateral symmetry. The illusion of a spiral arrangement would readily result from their larger numbers, five or six pairs in each cormidium, especially if combined with an obliquity of the rows. There is no ground for regarding the stem itself as spirally twisted, except slightly upon contraction.

The course taken by the combined processes of budding and growth in *Macrostelia* can now be summed up. In *Physophorids*

the bracts, when present, are arranged in lateral pairs on either side of a median series of polyps and gonodendra. In Calycophores the bracts are median, one to each cormidium, so that the triserial budding of Physophorids is reduced to uniserial in Calycophores. Moreover the buds in Physophores are separate from the start, whereas in Calycophores all the buds of each cormidium arise by subdivision of a single mother- or pro-bud, in which the various bud-rudiments or determinants have been, as it were, condensed. Budding in Calycophores is therefore more concentrated and less normal than in Physophores, so that, if one has been derived from the other, it is the Calycophore method that has been derived from the Physophore, and not vice versa.

But, while this difference is a point to bear in mind, the subsequent course of events is the same in both groups. Whether uniserial or triserial, the buds of each cormidium, as soon as formed, are immediately carried downwards by a marked excess of longitudinal over horizontal growth of the body in the zone of their formation, and a new set of buds promptly arises in their place, to be carried back in the same way by the ever-lengthening and scarcely broadening body.

This surely is no primitive state of things. The frequent use of the term *stolo prolifer* in connexion with the formation of this chain of buds has been greatly misleading, for the series differs radically from a *Salpa*-chain and similar proliferations in the fact that it is the parental body that extends and carries the buds along, and not a simple series of successive constrictions from a proliferating prominence. We have to think of the lengthening process as something quite distinct from the budding process. The body lengthens and carries the buds onwards for its own needs, which are clearly to spread its fishing tentacles and mouths through as great a zone of depth as it can, in order to increase its chances of catching scattered and elusive prey.

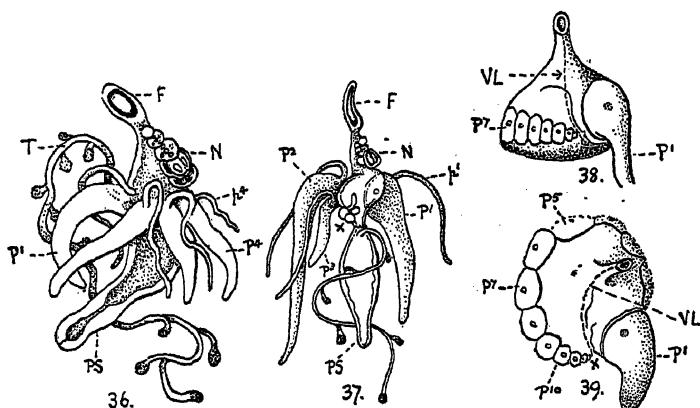
It is almost exactly the principle of long-line cod-fishing. With eight men in a boat, each lowering a separate 50-fathom line with a baited hook at every yard, the lines would soon be hopelessly entangled and the fishing ruined; but let each man lower his line in turn, after fastening it to the end of his prede-

cessor's, the result is a single free line of 400 fathoms with 800 hooks, each of which has an equal chance of catching a fish. So here the 'metameric' succession of cormidia, each attached to its predecessor, ensures more efficient fishing than 8, 16, or 24 cormidia separately suspended in a whorl round the Siphonophore body. The latter is the archaic method of the Brachystele, and of its human counterpart—good enough for shallow waters and the whiting fisherman, but with obvious limitations. If Claus and Haeckel had been fishermen instead of philosophers, they would never have dreamed that an oceanic Siphonophore would give up its Macrostele fishing-tackle for a hook-and-liner's Brachystele. Yet that is their theory, and in the next chapter we will examine it.

As an appendix to this section, to illustrate the nicety of a Siphonophore's adaptations for fishing, let me add this vignette from Claus's account (1879) of his marvellous '*Agalmopsis*' (*Lychnagalma*, Haeckel, Pl. xvi) *utricularia* from Messina. Each tentacle is buoyed up in a more or less horizontal position by a graduated series of tiny, but relatively large, floats, one beyond each battery of nematocysts (sacculus) in the middle of each lateral branch, and from each float no less than eight terminal stinging filaments radiate. Thus, as its discoverer relates: 'These filaments stretched out on every side (and at various levels) constitute in effect a kind of net spread all around the colony and ensuring the capture of every creature that penetrates its meshes.' How little we can understand such complicated little creatures as these until we have taken the measure of their special adaptations!

## 11. GROWTH AND SYMMETRY IN BRACHYSTELIA (Text-figs. 36-47).

On the current theory which we owe mainly to Claus (1860, 1878) and Haeckel (1888), the short-stemmed forms of Physophorid (Brachystelia) have been derived from long-stemmed Macrostelina (which includes all Calycophora as well as many Physophorids) by a secondary shortening and broadening of the stem. The tendency of the cormidial appendages to be disposed in whorls or a continuous spiral is attributed to a twist of the



TEXT-FIGS. 36-9.

*Physophora hydrostatica*. Larval and Young Colonies. *F*, float; *N*, nectocalyx; *P*<sup>1</sup>-*P*<sup>12</sup>, palpons (or their facets) in order of formation; *PS*, protosiphon; *p*, palpacle; *T*, tentacle; *VL*, ventral line, dotted; *X*, budding point.

Fig. 36.—Larval colony with four palpons, right-dorsal aspect. (Haeckel, 1869.)

Fig. 37.—Similar stage from left-ventral aspect. (Huxley, 1859, but with the missing protosiphon and tentacle restored after Haeckel.)

Fig. 38.—Ventral, and 39, aboral view of siphosome of a later stage with twelve palpons, all removed except the first (*P*<sup>1</sup>). The nectosome is truncated. (Based on photographs of Bigelow's, 1911.)

stem round an imaginary central axis. 'That they', says Haeckel, 'are often radially disposed depends wholly upon a secondary spiral twisting of the stem' (l.c., p. 9).

This theory of a twisted stem was originally held to apply to the nectosome of *Macrostelia* as well as to the siphosome of *Brachystelia*, until Chun showed that in well-preserved *Agalmids* and *Physophora* the stem bearing the swimming bells is perfectly straight, and that their biserial arrangement is produced by the bells bending alternately to right and left from their anchorage in the mid-ventral line (1898, p. 324, figs. 1-8). Even the great tiara of *Forskalia* is built up in this way.

The similar-looking swimming column of the Calycophore *Hippopodius* seems at first sight to be an exception to this rule, since its nectocalyces are demonstrably arranged upon a spiral axis (Haeckel, l.c., Pl. xxix). But this axis is not a part of the original stem, since, as was shown earlier, the whole of the aboral region atrophies. Here each new bell arises on the foot-stalk of its predecessor, and the series of out-growing stalks constitutes the spiral axis or 'Scheinstamm', as Chun called it (1896 b, fig. 11). Lacking a float to keep it upright, this composite bell-stalk bends completely over from the weight of its pendent nectocalyces, and twines round the siphosome for support—a peculiarity which points strongly to the conclusion that the absence of a pneumatophore in Calycophora is a case of secondary loss, not of primitive simplicity. In *Physophores* the budding point moves upwards with the growth of the stalk of the pneumatophore; in *Calycophores* the nectocalyces are recruited from below.

But a true exception to the rule is provided by the *Brachysteles Rhodaliidae* ('*Auronectae*', Haeckel), in which the nectocalyces, instead of mounting above one another along the stem of the pneumatophore, range themselves round its base to form a corona. As they all arise from the ventral budding tract, and the corona is interrupted dorsally by the aurophore (Haeckel, Pl. iv, 16; Bigelow, xxiii, 6 and 7), it is clear that new nectocalyces join the corona from each side of the ventral line, and that the diameter of the stem is increased *pari-passu*. Here at



any rate the corona is no product of a spiral twist of the stem, but of symmetrical bilateral budding and transverse growth of the body wall from the ventral line, the oldest bells being dorsal, the youngest ventral. If the same method of growth applied to the whole body, the cormidial buds below would also be arranged in parallel coronas of gonodendra and siphons. Actually they are arranged in a continuous dextro-tropic (clockwise) spiral, (Haeckel, fig. 17; Bigelow, xxiii, 11), the formation of which can best be studied in *Physophora* and *Discolabe*.

In *Physophora* (also *Discolabe*, Haeckel, Pl. xix-xx) the normal type of nectosome is combined with an asymmetrical reniform siphosome, which bears a single, or an imperfectly double, whorl of tentaculate palpons around its margin and, below this, two parallel whorls of gonodendra and siphons. The siphosome has been regarded as an inflated spirally twisted stem. The best case for this view was presented by Claus (1860, 1878) and illustrated by exact figures of two specimens from above and below. 'Der sack-förmige Polypenstock', he says, 'auf einen einfachen in Centrum verwachsenen Spiralbogen zurückzuführen ist.' The siphosome in his quaint first drawing (1860) has the outlines of a flounced crinoline split on the ventral side by a furrow (Claus's 'Naht') which contains the budding line on its right edge. Each flounce corresponds to a cormidium, consisting typically of a palpon above, a gonodendron below it, and a siphon lowest of all. He compares the regular succession of these trimorphic groups with that on a *Diphyid* stem. 'Assume', says Claus, 'a severely shortened and greatly widened stem, on which the groups of appendages are closely squeezed against one another, then the similar types of appendage will arrange themselves in rows, each forming its own circle.'

Claus ignored the fact that the appendages of a *Calycophore* arise as a single longitudinal series: bract, gonophore, tentacle, polyp; bract, gonophore, tentacle, polyp, &c. The spiral winding of such a uniserial stem, after suppression of its internodes, would merely change its direction from longitudinal to horizontal. No amount of 'squeezing' would transform its single polymorphic series into homogeneous parallel coronas as we see

them in a *Physophora*, 'each type forming its own circle'. Moreover the theory is at variance with the actual mode of development.

In the first stage the larva is monogastric, with no secondary siphons or gonodendra. It floats by its pneumatophore, its nectocalyces are developing but not yet functional, and its first palpons develop one after another to form the first corona. Haeckel (1869) reared *Physophora* from the egg to this stage, saw the first palpon arise on the ventral side, and move sideways to the right; and saw a second, third, and fourth palpon arise one after the other in the same place, each pushing its predecessor farther up the right side until the oldest reached the dorsum and protruded through the median dorsal cleft of the provisional larval bract (i.e., figs. 21 and 23). At this stage his oldest larvae died, each possessing a half-ring of palpons on its right side, and none on its left. He laid stress on the fact that the larval tentacle, arising as usual in the ventral line, remained in that position, while the palpons moved successively away from it. It is remarkable that no larva of *Physophora* with a complete ring of palpons has yet been found. Haeckel fished one from the sea which was almost identical with the oldest specimen he reared (Text-figs. 24 and 25), and the larva figured by Huxley was in the same stage of development, completely destitute of palpons on the left side. His drawing (1859, Pl. viii, 2), from the bare side, is the only one yet published which shows the condition of the stem. The siphon and its tentacle had clearly broken off during capture (a frequent experience with these sensitive creatures—see Sars, 1877), and Huxley's figure shows the jagged line of fracture. In my copy (Text-fig. 37) I have restored them from Haeckel's data, placing the tentacle on the ventral side of the siphon near its base.

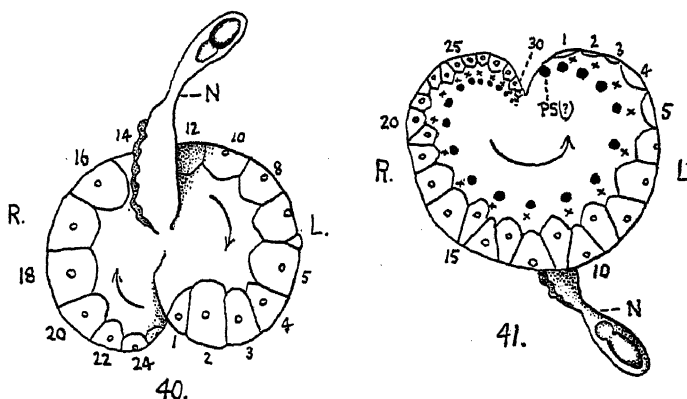
It will be seen that the stem of the siphosome retains its vertical position, but is dilated in the zone of the palpons, and tapered below to form the neck from which the protosiphon is suspended. If the stem as a whole had been 'twisted', the tentacle base would now be out of sight. It is asymmetrically bent above towards the dorsal side, which now

carries the first and stoutest palpon, but this is just what would follow from a predominant growth of the right side simultaneously with the proliferation of the palpons. A continuance of the same onesided process of proliferation and growth would bring the dorsal palpon round to the left side, and ultimately into close proximity to the ventral starting point.

In a later stage photographed by Bigelow (1911, Pl. xvi, my Text-figs. 38 and 39) from the 'Albatross' collection, the stem has fully attained its discoidal character and is surrounded by an equatorial ring of some 10-12 palpons, which increase regularly in size in dextral sequence from the budding zone. Secondary siphons and gonodendra have arisen beneath the ring of palpons, but their exact number and relations were not described.

To this stage, however, some of Kölliker's young colonies clearly belong. Their sizes ranged from  $\frac{1}{2}$  in. to  $2\frac{1}{2}$  in. in length, exclusive of their long tentacles. They possessed a 'full crown' of seven to ten palpons, mostly eight or nine, and two to five siphons on the underside of the conical siphosome. His figure (1853, Tab. v, 1) shows nine palpons and three siphons. Immature gonodendra were observed clearly in the larger specimens. Whether the protosiphon persists or not is left uncertain, but these data are sufficient to show that the larval whorl probably includes at least six palpons, possibly one or two more, before the secondary siphons and gonodendra begin to appear.

The final stage described and figured by Claus (1878) and Sars (1877) is that described at the head of this section—an asymmetrical reniform disc, fringed around its convex margin by parallel horseshoe-shaped cycles of palpons above, siphons below, and gonodendra between the two, all of them interrupted by the ventral groove, which now clearly divides the disc into prominent right and left lobes. The right (nascent) lobe begins ventrally with a crowded line of young buds curved around its apex; the left (senescent) lobe ends opposite this apex in a blunt prominence carrying the oldest palpon. The upper surface of this lobe is definitely higher than that of the right lobe (Sars, l.c., figs. 1-4; Claus, fig. 3), a circumstance which explains a striking feature in Gegenbaur's excellent figure of a living colony (1860; Taf 30, fig. 32), in which the oldest palpon is seen to end



TEXT-FIGS. 40 AND 41.

*Physophora hydrostatica*. Siphosome of adult colony after removal of all appendages (after Claus, 1878, simplified). *N*, nectosome with float; *p* (1-31), palpon-facets numbered by age; *PS*, proto-siphon (?); *R*, right; *L*, left. Arrows show the one-sided direction of growth (dextral) from ventral line. Siphons ●●; Gonodendra ××.

Fig. 40.—Upper surface, showing marginal series of palpon-facets. The youngest (*P* 27-31), and those of a lower series, are invisible from above.

Fig. 41.—Lower surface, diagrammatically extended to show the full series, as well as the points from which the siphons and gonodendra arise.

the series abruptly at a higher level than the younger palpons in the whorl. The earlier development of this feature has been traced above from Huxley and Bigelow's figures (my Text-figs. 36-9).

Neither in Claus's nor in Sars's figures is there any indication of any further extension of the coil than this. In other words, the buds of a *Physophora* colony form three parallel, ever-widening, but uncompleted rings, one above the other, the ends of which in each case approach one another, but never meet. Proliferation and growth broaden each ring, and bulge it on either side to a reniform shape, but the distal (oldest) end is not produced into a spire.

Although the cycles of gonodendra and siphons run parallel courses around the discoidal stem below the palpons, there is a numerical peculiarity to be noted as regards the palpon cycle, to which both Claus and Sars have already drawn attention, but which Haeckel dismissed as an 'accident' (p. 258). In the more regular of the two prepared specimens which Claus figured (figs. 3 and 4; my Text-figs. 40-1) the palpons, as marked by their facets of articulation, form a single marginal row round the greater part of the left (senescent) lobe, but a double row round the whole of the right (nascent) lobe, except close to the budding centre. Sars also figures a double row in the same lobe (l.c., Tab. V, figs 1-6), without defining its extent. In each case the extra row consists of small triangular facets on the lower side of the large pentagonal marginals, and alternating with them. The upper row of the right lobe is continuous with the single row in the left lobe. Siphons and gonodendra are present in equal numbers, about twenty of each, but the total number of palpons is about thirty, of which rather more than half (actually seventeen) form the marginal series of large facets. In the densely crowded initial part of the sequence next the budding zone, the siphons and palpons appear to be equal in number, so that their numerical disparity in the disc, for which no explanation has yet been given, is probably not due to any inequality in their rates of proliferation. It should be noted, therefore, that the difference (about eight to ten, but exact numbers cannot be given) corresponds closely with the number of palpons in the

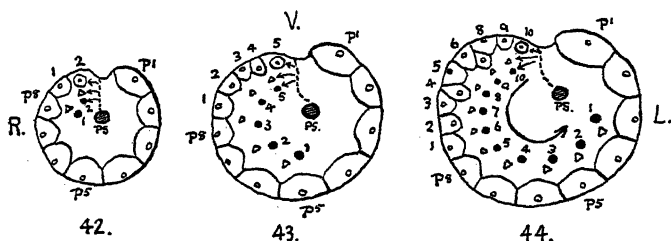
larval whorl which, as shown above, is probably completed before the appearance of gonodendra and siphons. As all additions are made from the ventral line, an important conclusion follows. When the larval circlet of palpons has been completed, the under surface of the disc must be free of all appendages except the protosiphon, which has been pushed to the left by the preponderant growth of the right side. Consequently the trimorphic groups ('cormidia'), which then begin to appear, will not have equal freedom of movement for their members. The gonodendra and secondary siphons, as they leave the budding zone, will have an open space before them, but their palpons, following those of the larva around the margin, will find their line of advance already occupied by their predecessors. Thus the one-sided dextral growth which accompanies their proliferation carries the gonodendra and siphons freely into unoccupied territory, while their palpons, pressing in single file against the rear of their predecessors in the right (nascent) lobe, are squeezed, as they grow, into a zigzag double series round the margin (Text-figs. 42-4). The variability of their arrangement described by Claus is doubtless due to different degrees of pressure in different individuals, consequent upon inequalities of nutrition and growth.

In any case it will be seen that Haeckel's statement that the cormidia of Physophoridae preserve a 'perfectly ordinate' character is incorrect. The gonodendra and siphons certainly keep and move together, but, in their rotation round the disc, the earlier pairs of these must gradually push past the palpons which were budded with them, and, on entering the empty region of the left lobe, must eventually come into a new alignment with the larval palpons already present around its border. Thus the arrangement of appendages in the siphosome of *Physophora* is not the result of the spiral twist of an ordinate Macrostele stem, but of one-sided (dextral) proliferation and growth from three successive zones of the budding line. According to Haeckel the peripheral margin of the disc, to which the cormidia are attached, is the ventral median line of the twisted stem (p. 265). On the contrary, the cormidial line is drawn by the succession of larval palpons at right angles to the stem, as a horizontal ring around it, the stem itself remaining

straight and untwisted. Gonodendra and siphons follow, tracing two more lines parallel with the first. Therefore the whole three-fold cormidial sequence is at right angles to the stem, not parallel with it.

Some new questions arise when we pass from the more familiar *Physophora* to Haeckel's '*Discolabe quadrigata*' from the Indian Ocean, which competent authorities have regarded as generically, and even specifically, identical with the almost ubiquitous *Physophora hydrostatica* (Schneider, 1898, p. 126; Bigelow, 1911, p. 292). If these identifications could be accepted, *quadrigata* would furnish an additional or fourth stage in this summary of *Physophora* development, for the cormidial coil, which forms a single ring in *Physophora*, is produced in *Discolabe* into a typical two-whorled spire beautifully drawn by Haeckel (Pl. xx, fig. 12). But neither Haeckel nor his successors noticed that this spire has a sinistral (counter-clockwise) coil, instead of the dextral (clockwise) coil that has characterized every *Physophora* hitherto described, adult or larval. The astonishing thing is that in his text Haeckel referred explicitly to 'figs. 9-13' on this plate for confirmation of his statement that 'the spiral turning in the siphosome of all *Discolabidae* (= *Physophoridae*) seems to be dextrotropic' (p. 258). Yet of these five figures all but one (fig. 13) show sinistral ('laeotropic') coils! (This is but one of many similar lapses from accuracy which disfigure Haeckel's 'Challenger' Report when he deals with matters of Right and Left, Dorsal and Ventral, Dextrotropic and Laeotropic. They are in part, if not always, traceable to the 'promorphological' orientation of Siphonophores which he set up in his youth (1869) and continued in his 'Challenger' Report, with the mouth above, and the aboral extremity below, thereby turning the natural Right into Left, and Left into Right, in complete disharmony with his illustrations, which are always the right way up. In these the artist was wiser than the philosopher.)

The single dextral specimen represented in Haeckel's fig. 13 was a small colony, so similar to Bigelow's photograph copied in my Text-figure that I have no hesitation in claiming it as a representative of the same species, *Physophora hydro-*



TEXT-FIGS. 42-4.

*Physophora hydrostatica*. Oral surface of denuded siphosome in successive stages of unilateral (dextral) growth and cormidial proliferation. Ventral side (*V*) uppermost. (Original diagram.) Young palpon,  $\odot$ . Gonodendra,  $\triangle \triangle \triangle$ . Siphons,  $\bullet \bullet \bullet$ . Ventral Line in hilum of reniform disc, - - - -.

Fig. 42.—Early post-larval colony with two cormidia (1, 2). Larval palpons (*P* 1-8) already surround the disc. They are recruited by the cormidial palpons, while the siphons and gonodendra at a lower level are carried past them.

Fig. 43.—Stage with five cormidia (1-5). Increased pressure on the marginal palpon-facets. The siphons and gonodendra continue to advance.

Fig. 44.—Stage with ten cormidia (1-10). The cormidial palpons round the margin are squeezed into a double row. Their siphons and gonodendra nearly surround the disc. The protosiphon (*PS*), displaced to the left by the preponderant growth of the right side, will soon head the procession of siphons in the left (senescent) lobe (cf. fig. 41).



statica. It would appear that Haeckel mixed up two distinct species in his account of *Discolabe quadrigata*—a true *Physophora* with dextral coil (fig. 13) and a new form with counter-clockwise spire, to which the name *Discolabe quadrigata* may be provisionally restricted (figs. 9–12). Possibly the ‘*Dicymba*-like’ young colony from the South Atlantic, represented in Haeckel’s Pl. xix, fig. 8, which also shows a sinistral sequence of palpons, may be an early stage of this type.<sup>1</sup> Following Haeckel’s account in other respects, the revived genus may be distinguished by its quadriserial nectosome, its sinistral siphosome terminating below in a true spire, and by its single series of marginal palpons and their facets.

The extension of the *Physophora* ring into a continuous spire involves no change in its theoretical interpretation, and but minor changes in the form of the colonial body. Upon reference to Haeckel’s Pl. xx it will be seen that in the sinistral *Discolabe* (figs. 9–12) the nascent line of palpon facets is visible almost to its origin, whereas in the dextral *Physophora* (fig. 13; also Claus, 1878, iii, 3, &c.) it is completely hidden within the deep groove of the disc by the overhanging senescent lobe. In other words the ‘senescent’ lobe, which is on the Left side and elevated in *Physophora*, is on the Right side and depressed in *Discolabe*, thus enabling the older palpons to turn the corner of the lobe and move inwards on its lower face, where the spire is continued (figs. 10 and 12). The reduction of growth-pressure on the marginal row of palpon facets, which must result from this extension, probably explains the simplicity of the series in *Discolabe*, as compared with the tendency towards duplication proximally in *Physophora*. Incidentally Haeckel pointed out that the appendages of the inner coil are older than those of the outer, and gradually

<sup>1</sup> The other larvae on this plate (figs. 5–7) are apparently touched up copies of the author’s original figures of *Physophora* (1869, figs. 15, 20, 22, and 23) and of very doubtful validity. The statements about them on p. 261 flatly contradict his original statements as to ‘ventral tentacle’ and ‘dorsal cleft’ without any explanation. Lacking corroboration, both should be ignored.

diminish in size. This means that they undergo degeneration, with the loss of their articulating facets, as they turn the ventral corner of the senescent lobe (Text-fig. 45).

A third type of Brachystele is seen in *Anthophysa*, already referred to as a connecting link between Cystonects and Physonects in the structure of its huge float which, with its pericystic outgrowths, entirely fills the stem (Text-fig. 45). This is a stout conical vesicle gradually expanding below into a boat-shaped disc with its long axis dorso-ventral. An extension of the ventral region forms the actual floor of the disc, from which a median row of siphons depends, the most dorsal of which is doubtless the original protosiphon. In all these features *Anthophysa* shows significant resemblances to *Physalia*. Above, however, below the apex of the pneumatophore, it is surrounded by a great corona of paddling bracts, supported at the ends of a series of muscular ridges which radiate, with varying degrees of obliquity, from the ventral budding cone of the nectosome. Below the bracts is a second oval corona of much less numerous hermaphrodite gonodendra. Large numbers of small non-tentaculate palpons accompany the gonodendra, and also extend across the floor of the disc in groups that alternate with the siphons (Bigelow, 1911, xxiii, 5). The ventral line above the youngest siphon is marked by a crowded series of young cor-midial buds, five or six abreast (Haeckel, Pl. xi, 9).

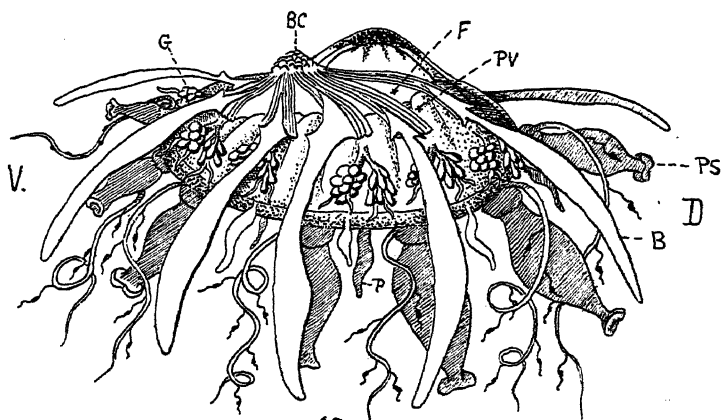
The corona of bracts arches over the whole colony, as in its close ally *Athorybia*. The ridges which bear them are muscular folds of ectoderm and mesogloea, each traversed by a fine canal of endoderm continuous with that of the bract (Bedot, 1904, i, 13-15; Bigelow, 1911, xx, 7 and xxiii, 3). The course of this canal has not been described, but from Bigelow's figure it would seem to run directly into the body through the distal part of the ridge. The uppermost ridges are longest, and extend towards the dorsum, the middle ridges run to the flanks, and the lowest and shortest, which are of course the youngest, support the ventral bracts (Haeckel, xii, 7-9). They are arranged in bands of four to six or more, separated by bare intervals, and the number of bands corresponds with that of the gonodendra below them. The bracts accordingly are four to six times as numerous

as the bands and the gonodendra, and these on each side correspond in number with the secondary siphons.

From this equality in their numbers the bands (or groups of bracts), the gonodendra, and the secondary siphons might be expected to form the same number of parallel, vertical series, and this alignment is actually shown by the younger zooids of each type, i.e. those nearest the budding line; but further dorsally the siphons lag behind this alignment, probably because of the protosiphon in front of them, and come to alternate with the others, their place being taken by the alternating groups of palpons (Bigelow, xxiii, 5; my Text-fig. 45). Apart from this minor dislocation the co-ordination of the appendages of nectosome and siphosome is the more remarkable as the buds are derived from at least two different centres of proliferation. It implies the existence of a common factor of co-ordination, which we shall show to be contained in the common mode of growth.

Haeckel misunderstood the distribution of the palpons, thinking that they formed a separate corona between the bracts and the gonodendra—a mistake which Bigelow corrected. He also likened the cormidial composition 'to that of *Physophora* and the other *Discolabidae*, the cormidia being ordinate and arranged symmetrically in a flat spiral line which is twisted around the flat and broad base of the shortened vesicular stem'. The symmetry is clear, but the cormidia (following Bigelow's elucidation) are not ordinate, but 'dissolved'; the gonodendra form a ring, not a spire; and the straight line of the siphons (which Haeckel himself noted) neither accompanies the ring nor is 'twisted' round the stem. Surely *Anthophysa* is no type to be invoked in support of the theory of a twisted stem.

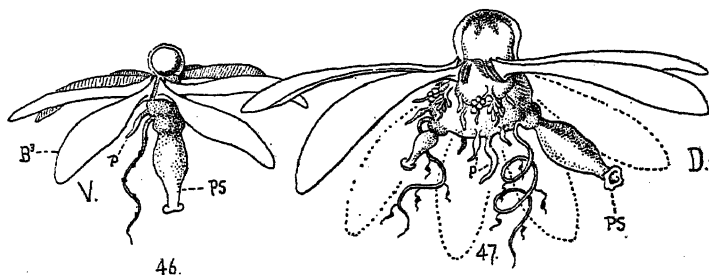
The explanation here submitted is essentially the same as that applied above to the case of *Physophora*, but simpler because of the complete bilateral symmetry of *Anthophysa*. The corona of bracts is obviously produced by bilateral proliferation and growth from the ventral line, the ectodermal ridges leaving a persistent record of the direction of their growth after each proliferation. Taking for example a case in which each band of bracts includes four ridges, the first or larval stage will be



45.

TEXT-FIG. 45.

*Anthophysa formosa*. A large colony schematically represented from left side. (Based on Bigelow, 1911, but with omission of numerous palpons between and below the gonodendra, reduction of bracts to one from each band, and relative reduction in size of all siphons and palpons. Original.) *B*, bracts; *BC*, budding cone of bracts ('anterior blastocrene'); *F*, float; *G*, gonodendra (hermaphrodite); *P*, palpons; *PS*, protosiphon; *PV*, pericystic villi and septa; *D*, dorsal; *V*, ventral.



46.

47.

TEXT-FIGS. 46 AND 47.

*Anthophysa formosa*. Hypothetical larva and young colony to show the probable course of development by successive increments of ventral proliferation and ventro-lateral growth. Lettering as in fig. 45.

Fig. 46.—Larva as in *Athorybia*: four pairs of paddling bracts attached ventro-laterally. Protosiphon only, followed by the first median palpon.

Fig. 47.—Next stage with a cormidial siphon ventrally, in line with the protosiphon dorsally, two pairs of lateral gonodendra midway, and two pairs of bract-bands (each with four bracts) over the enlarging float, together with many palpons.

marked by the development of four bracts on each side (Text-figs. 46-7). An interval of growth follows, predominantly from the ventral side, and the basal ridges are extended centripetally (towards the 'cone'), thereby increasing the muscularity of the bracts. Then a new batch of bracts is proliferated on the ventral side of the first, and alternating processes of proliferation and growth are repeated, each new period adding an additional band and four more bracts to the corona on each side.

Simultaneously with these processes in the nectosome, after the first or larval stage, the cormidial centre proliferates a lateral pair of gonodendra and a median siphon, followed by an interval of growth, which carries all three appendages dorsally—the gonodendra laterally and the siphon obliquely backwards. During this period an indefinite number of palpons<sup>1</sup> is produced on either side, and these, according to the growth gradient in which they occur, are carried away, some more or less transversely, others backwards, either obliquely or in the middle line after the last-formed siphon.

Thus every zooid of each class, cormidial and locomotive, arises as a separate bud from the ventral tract, and takes part in a general concerted movement towards the dorsum with complete bilateral symmetry—bracts above, gonodendra and siphons, intermixed with palpons, below. The effect of the combined processes of ventral budding and lateral ventro-dorsal growth of the body-wall is to bring about a zonary distribution of the different kinds of zooid almost identical with that of a *Velella* or *Porpita* colony, but with bracts in place of tentacles above, gonopalpons (= 'blastostyles') with their gonophores in the middle, and siphons below—nectocalyces being absent in both cases. *Anthophysa* is thus almost unique among *Siphonanthae*, both in the completeness of its bilateral symmetry and in the extent to which it rebuilds a radial arrange-

<sup>1</sup> Bigelow states that 'New ones in various stages of growth are to be seen in various regions' (l.c., p. 298), and in *Dromalia* attributes the formation of whole cormidia to the presence of secondary buds near the bases of siphons far from the ventral line (p. 309). Such exceptions to the 'law' of *Siphonanth* budding would be of first-class importance if fully substantiated.

ment of its zooids—in both respects recalling the *Corymorphine Branchiocerianthus*.

But, although I cannot demonstrate it, the real climax of this series will probably be found in the rare *Cystonect Epibulia*, described and figured in Haeckel's 'Monograph' (1888, Pl. xxii). The trunk of the siphosome is described as 'short, wide, and bag-shaped', and the 'ventral line' of appendages as 'circular or rather spiral'. But Haeckel admits that he was 'unable to examine closely the form of the central trunk and the attachment of the cormidia', and adds that it is 'probably similar to that of the Anthophysidae, Discolabidae and Nectalidae'. 'All I could observe', he says, 'was that it represented a shortly conical or ovate bladder, coiled up in a spiral, with a single dextrotropic turning.' This is essentially the same description as he gave of the siphosome of *Physophora*, so that a similar explanation of it is to be expected. But there are some differences.

On Plate xxii he figures various larval stages attributed to 'Cystalia', which is now generally regarded as merely the young monogastric stage of *Epibulia*. In fig. 4 a larva is drawn from the right side to which a 'corona of buds (palpons)' is attributed. The half-corona of six palpons shown reveals a steady increase in the size of the palpons from venter to dorsum, and the other half doubtless showed the same sequence. If the whole corona were the product of a one-sided proliferation, as in *Physophora*, some of the larger palpons of the left side would undoubtedly have been visible near the base of the ventral tentacle. It is, therefore, probable that the whole corona is formed by a symmetrical proliferation on both sides as in *Anthophysa*. This inference is confirmed by the structure of the later stages (figs. 5 and 6), in both of which a crowd of young buds is drawn on the ventral side, and the largest palpons are seen laterally and dorsally, with obvious bilateral symmetry. Similarly, there can have been no kink or hilum in the 'ovate bladder' bearing these appendages, as there is in *Physophora*, or Haeckel would have noticed it, and would not have described the 'ventral line' as 'circular'.

The inference is accordingly that the cormidial buds, as well

as the palpons, are proliferated equally on both sides of the true ventral line, and, by their movement dorsally at right angles to this line, form bilaterally symmetrical rings, as do the nectocalyces of *Rhodalia* and the bracts and gonodendra of *Anthophysa*. It is thus possible, even probable, that *Epibulia* possesses a complete biradial symmetry, which differs from that of *Anthophysa* in having a basal ring of siphons around the protosiphon like the peristomial ring of secondary polyps ('blastostyles') of *Disconantha*.

Whether this be so or not, I cannot but think that a perfect biradial symmetry is the primitive condition of Siphonanthus, and that all the other conditions which in various degrees have seemed to be leading up to it, are in reality so many deviations or declinations from it. In the case of *Anthophysa*, for example, there is a manifest peculiarity in its obliquely extended float amply sufficient to account for the mid-ventral multiplication of its siphons. In effect the colony floats like a *Physalia*, with its ventral side largely horizontal instead of vertical, and the proliferation of its siphons has been affected accordingly. *Epibulia* on the other hand retains the primitive vertical orientation, conducive to radial symmetry; but a symmetry progressively built up, by proliferation and growth from the ventral line, exactly as in *Branchiocerianthus* (fig. 48).

As cited above, Haeckel also included *Nectalia* among his *Brachysteles* with a spiral corm, but from lack of exact data this can be neither confirmed nor denied. Its outstanding feature, however, is the unique differentiation and symmetry of its bracts. These are few (eight) in number, of three different sizes, and arranged around the body with a quasi-cruciform symmetry. They constitute a corona which is 'raised and subhorizontally expanded in the quietly floating corm, whilst they form a closed bilaterally compressed calyx in the rapidly swimming animal' (p. 250). There is a pair of short leaf-shaped bracts in the plane of the nectocalyces (i.e. right and left of the budding line), a pair of long sword-like bracts at right angles (i.e. dorsal and ventral), and a quartet of intermediate-sized bracts interradially placed between the four perradials.

(Haeckel wrote before Chun (1898 b) had shown that the pairs

of nectocalyces in a biserial column were not dorsal and ventral, as was then generally believed, but right and left (cf. p. 157), so that the terms 'sagittal' and 'frontal' in his account need correction. The 'lateral compression' he speaks of is really a compression in the sagittal plane, which bears the long bracts, while the short ones are right and left, at the ends of the longer axis, i.e. the colony is broader from side to side than from front to back.)

It will be realized that an appendage in the middorsal line looks like a breach of the law of ventral budding, which is even aggravated by the bract being a member of a diagonal pair in the sagittal plane. Yet precisely the same relations of the four perradials were observed by Bigelow (1911) in a younger colony of the same species, the only other specimen which has been adequately described. These relations must therefore be accepted as described.

Haeckel's description of the siphosome is almost identical with that he gave of *Physophora*: 'the trunk of the siphosome is shortened, vesicular, and horizontally expanded in form of a spiral bladder' (p. 260). For the *Discolabidae* it was: 'vesicular, much shortened and inflated, and coiled up spirally in a sub-horizontal direction . . . as in the *Nectalidae*' (p. 258). He adds a significant contrast with the condition in the *Agalmidae*: 'The trunk of the latter (i.e. *Discolabidae* and *Nectalidae*) possesses therefore permanently about the same shape which the trunk of the former (i.e. *Agalmidae*) exhibits only in the state of the strongest contraction' (p. 258). On the other hand he gives a separate figure (xiii, 3) of 'the trunk alone after detachment of the appendages', and draws it as an upright spindle-shaped vesicle bearing a straight vertical series of some fifteen to sixteen 'buds of siphons and palpons' along the ventral line! No conflict could be greater, and the labelling of his drawing of the full contracted colony (fig. 2) prohibits any sure interpretation which would reconcile one figure with the other. In fig. 2 the nectosoma is seen to be contracted with a dextral twist above a sinistrally twisted coil bearing vertical stripes. This might well be his 'spiral bladder' if he had not marked the stripes 'nb', which means 'nectocalyx buds', which they cannot possibly have been! No bract attachments are marked as such in either figure.



Schneider (1898, p. 124), convinced of the systematic affinities of *Nectalia* with Agalmidae by various details of structure, cut the Gordian tangle by throwing over Haeckel's words and pinning his solution to the very dubious fig. 3, which, for my part, I imagine must have been not the drawing of an actual preparation, but a diagrammatic sketch of what Haeckel thought the sinistral coil of fig. 2 would look like if 'untwisted'. Schneider interpreted the apparent whorls of bracts, palpons and siphons as a superficial consequence of the spiral twisting of an Agalmid stem, shorter and more inflated than usual, exactly as his master Claus had interpreted the structure of *Physophora* forty years before, except that Schneider assumed the stem of *Nectalia* to retain its Agalmid flexibility and to display the whorled appearance of its appendages only on contraction.

Schneider's theory, though favourably regarded by Bigelow on systematic grounds, raises more puzzles than it solves. The development of the bracts in diagonal pairs recalls the arrangement described above (p. 153) in the case of *Agalma okeni*, &c; and the marvellous carapace of Haeckel's *Agalma 'eschschoitzii'* shows what can be accomplished by cormidial bracts suitably fixed. If it were possible to eliminate all the lower bracts of that species, and to differentiate the topmost series by contrasts of size on a radiate plan, *Nectalia* might be its next of kin. But Bigelow's specimen vetoes any such ideas.

As we have seen, the process of budding and growth in *Macrosteles* carries the cormidia downwards as fast as they are produced, and the newest cormidia are those at the top of the series. Haeckel's specimen of *Nectalia* possessed eight bracts and four siphons. Bigelow's young colony possessed only two siphons, yet it displayed the four perradial bracts in the same form and in the same positions as in Haeckel's older specimen possessing two additional cormidia, and the four new bracts in Haeckel's specimen were small and undifferentiated. Clearly the four perradial bracts in a young colony like Bigelow's are not carried downwards with an increasing number of cormidia as they should be upon Schneider's Agalmid hypothesis. In other words the bracts of *Nectalia* are precormidial in position, and

presumably homologous with the bracts of the nectosome of *Athorybia* and *Anthophysa*.

As regards their symmetry, Bigelow shows that the small pair are situated in line with the nectocalyces and above the level of the blastocrene of siphons, and that both pairs are supported by the usual muscular pedicels. It is therefore probable that the cruciate symmetry shown by the bracts is explicable in the same way as the cruciate symmetry of nectocalyces in a quadriserial nectosome, of which both the *Physophoridae* and *Nectalidae* furnish examples (*Discolabe* and the *Sphyrophysa* of Agassiz), viz. by adaptive bendings of their pedicels from the ventral budding line. Fixed in this way, and not subject to the perpetual shift backwards of cormidial bracts, their differentiation presents no special difficulties. Haeckel's lively account of the marvellous swimming powers of *Nectalia*, and its skill in avoiding obstacles, renders it probable that the long bracts, which he compared to 'lee-boards' in a sailing boat (mistranslated as 'swords', p. 254), may be a sensitive mechanism adaptive to that end.

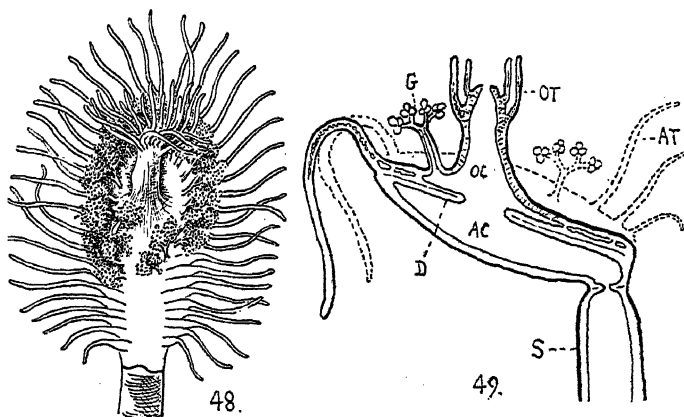
Bigelow's specimen, in spite of his careful study, was too small, and its small appendages were too crowded, to throw light on the problem of the siphosome; but indirectly, by helping to clear up the nature of the bracts, it has shown that in all probability the siphosome will ultimately prove explicable on the same lines as those of *Physophora* and *Anthophysa*.

## 12. GENERAL CONCLUSIONS (Text-figs. 48-57).

The advantage of an occasional stock-taking of our knowledge lies in the fact that, in addition to clearing the shelves of dusty relics, it serves to define the nature and extent of the gaps, so that efforts can be made deliberately to fill them up. The history of Siphonophores, necessarily proceeding piecemeal, and often based on scanty or ill-preserved material, has been rich in hasty theories, doctrines, and generalizations, but these have been adequately dealt with in my earlier chapters (§§ 1, 2, 5, 10, and 11). Here I will sum up the more general results of my survey, referring to particular chapters for explanatory matter and details of evidence.

Relations have been established between Siphonophora and Anthomedusae which are certainly closer, and probably firmer, than any previously advanced. Woltereck's larva of *Velella* (§ 5) contained evidence (in its mesogastric septum and subsequent aboral plexus) of an intimate relationship of Disconanthae with the Corymorphine group of Gymnoblasic Hydroids, and incidentally reveals *Pelagohydra* as something very near the ancestral stock—a pioneering effort of that stock to achieve a free-floating existence. But *Corymorpha* and *Pelagohydra* are non-colonial monogastric forms, and presumably only side twigs of the sessile colonial stock that gave rise to Siphonophora, while *Tubularia*, apparently their closest colonial relative to-day, is in several respects too specialized to illustrate adequately its original characters. On this I will add some remarks farther on (p. 187).

On the other hand, the gap between Disconanths and Siphonanth, which Schneider admitted to be 'himmelweit', has been materially reduced, negatively by removing the grounds on which the Calycophora have so long been regarded as primitive, and positively by showing the assimilation of the Brachyste group to *Corymorpha*'s next-of-kin, *Branchiocerianthus* (Text-figs. 48-9), owing to their common bilateral mode of growth from one side. This assimilation means that the short-stemmed Siphonanth, with their buds arranged in whorls over the sides of the body, are more primitive than the long-stemmed forms, in which the buds form a single ventral series along a greatly attenuated stem. The different symmetries of Disconanth and Siphonanth are thus no longer prohibitive of close relationship, as seemed probable when my study began (§ 5). Nevertheless, anatomical divergences between Disconanths and Siphonanth remain as regards the structure of the float, the storage of nematocysts, and the simple or ramified coelenteron; the polymorphism of Siphonanth cormidia closely recalls that of the reproductive branches of certain Gymnoblasic colonies; and an affinity with a diffusely tentaculate stock of that group is suggested by various details in the structure and life-history of *Myriothele* (§ 8). Let us take these points in order.



TEXT-FIGS. 48 AND 49.

*Branchiocerianthus* (*Monocaulus*) *imperator*, deep-water Corymorphine with ventral proliferation and bilateral symmetry. *AC*, aboral chamber; *AT*, aboral tentacles; *D*, diaphragm; *G*, gonophores; *OT*, oral tentacles; *S*, stalk.

Fig. 48.—Oral view of horseshoe-shaped series of gonophore-clusters and aboral tentacles recruited from below (Mark, 1898).

Fig. 49.—Diagrammatic sagittal section of disc, showing diaphragm partly fused with peristome (original, after Miyajima, 1900, modified).

The float of *Disconanths* differs from that of *Siphonanth*s in being composed of concentric chambers, produced below into fine tubular 'tracheae', and in lacking a recognized gas-gland. That of a *Siphonanth* is a simple vesicle, except for the secondary outgrowths of its gas-gland (§ 1). The latter, as suggested by Korschelt and Heider (1890, p. 44), could readily be derived from the invaginated 'Fussplatte' or sucker of an *Actinula* larva, especially if, as in *Myriothela*, the habit of fixation by this glandular disc was being given up (§ 8). In such a case, in tidal waters, the larva could easily fill its sucker with air, and float away like a *Minyad* on its adventurous voyage, carrying with it its inherited cargo of potentialities, and using its tentacles as paddles. But the arenicolous *Corymorphines* have no sucker or adhesive disc, being rooted in sand or mud by a multitude of fine rootlets. In the great *Monocaulus imperator* of the 'Challenger' (= the *Branchiocerianthus* of Mark and Miyajima) these are concentrated round a terminal cauline bulb, and in all *Corymorphines* (*Hybocodon*?) the normal chitinous perisarc is replaced by a soft thin pellicle, apparently subject to periodic ecdysis and renewal. If the rounded base of a *Corymorphine* *Actinula*, while still floating, were to undergo invagination, the chitinogenous ectoderm, being now inverted within the body, would presumably in due course secrete a thin chitinous vesicle produced into everted rootlets with a similar chitinous lining—the two primary features of the *Disconanth* float. Periodic withdrawals of the epithelium from the vesicle, keeping pace with the general growth, would result in a succession of ring-like chambers around the primary cyst, together with corresponding annulations upon the inverted rootlets, as on the so-called 'tracheae' of the float. The eight 'radial chambers' with which Haeckel complicated the structure, have been shown by Bigelow (1911) not to exist. Thus the two kinds of float may have arisen independently in these two ways, although it is equally true that the simpler float of *Siphonanth*s (apart from its gas-gland) may be the result of a secondary simplification of the *Disconanth* float, though this is not the simpler explanation.

The second difference in regard to the storage of nematocysts

is bound up with differences in the arrangement of the tentacles in the two groups. Like the common possession of a float, the mere existence of special stores of nematocysts in the two groups marks some kind of relationship, though the differences in detail are profound. No Hydroid polyp has a compact basal store of nematocysts comparable with the basigaster of Siphonanthus, though the zones of nematocysts in the Actinula of Tubularia are suggestive (Lowe, 1926), and no Hydroid colony has a central reserve like the centradenia of Disconanthus. In Siphonanthus each polyp has its own heavily armed and highly extensile tentacle, while the Disconanth polyps have no tentacles of their own, and the aboral wreath of the colony, in spite of the huge centradenia serving it, consists of relatively feeble structures, barely differing from the tentacles of Hydroids. Until this puzzling resemblance and difference has been explained, we cannot say with any confidence whether the tentacles of either type are modified Hydroid tentacles or dactylozooids. It looks as if the two conditions were divergent modifications of some unknown *tertium quid*. I can offer no explanation, but there is plainly here a promising field for a crucial piece of research, which should be associated with a comparative study of the distribution and, if possible, of the migrations of the different kinds of nematocyst, on the lines so well begun by Weill (1934).

The third difference as regards the septation of the hydranth and the plexiform coelenteron of the aboral or cauline region is not fatal to the possibility of close relationship. When present, these characters naturally have a high systematic value, although in Hydroids, owing to lack of anatomical information on many forms, systematists prefer to use external characters more or less associated with them. That their absence does not preclude close relationship is readily seen by comparing Pennaria with Tubularia. Moreover, the aboral plexus in its origin was clearly associated with the formation of a pseudo-notochordal axis for the support of large polyps on exceptionally long upright stems, probably at a time when the hard perisarc was still restricted to short basal cups on a retiform stolon. The loss of the upright attitude, as in Myriothele and Hypolytus

(fig. 56), or suspension upside down from floating weeds as in *Tiarella*, would remove this function and might well result in complete loss of the endodermal complexity. The same argument applies to Siphonophores. In Disconanths the inherited plexus has been retained and even extended for the better nourishment of the massive tissues around and below the float; but in most Siphonanth's no such reason exists, and a secondary return to simplicity may have ensued. The massive body of the Rhodaliidae (Haeckel's 'Auronectae') is the one exception, and it is certain that the canalization of the coelenteron in that group is quite different and independently acquired. There is a sequence in the group from complete simplicity in *Archangelopsis* to almost complete canalization in other forms, and even in these the aboral region may remain as a large undivided hypocystic cavity (Bigelow, 1911, xxi, 7). On this character, therefore, we are again presented with the same alternative as in the case of the float: the Siphonanth condition is directly derivable from that of the simpler non-Tubularian type of Hydroid, or may be derivable from that of Disconanths, or of Corymorphines, by a secondary simplification.

The last of our differentiating points yields evidence of a more decisive character. In general the mere number of polymorphic types is of little moment for tracing relations, because nectocalyces and palpons are obviously modified gonophores and polyps, and could be derived from those of either Hydroids or Disconanths. Bracts also can be excluded, since, as I have tried to show, they are neither polyps nor nectocalyces in origin, but special modifications of persistent larval tentacles (§ 8). But the position of nectocalyces may be crucial. The cormidial nectocalyces of certain Calycophores are obviously modifications of their own gonophores, provoked by the need of extra locomotive power for their specialized 'Eudoxiae', while all those of the nectosome, being produced as buds from the larval body (oozoid), must represent the gonophores of pre-Siphonophore (i.e. Hydroid) ancestors, since no Siphonophore, whether Disconanth or Siphonanth, produces gonophores on the oozoid. Like the oozoid of Salps and Doliolids, it remains permanently sterile, and the sexual gonophores are all produced by its off-

spring, the secondary budded polyps and gonopalpons. Moreover, as the nectosome corresponds to the hydrocaulus of a Hydroid, when a stalk can be distinguished, and not to the hydranth proper (§ 9), this means that the Hydroid ancestors of Siphonantha, unlike *Tubularia* and *Corymorpha*, produced their gonophores on their stalks, and not on their hydranths. This condition in the oozoid is actually represented in *Pelagohydra*, the locomotive tentacles and gonophores of which suggest the immediate precursors of paddling bracts and nectocalyces; but for the nearest colonial type with diffuse tentacles and cauline gonophores we have to look away from the Tubularian towards the Coryniform Hydroids, from some unknown stock of which the long-stalked and highly specialized Tubularians doubtless arose. A direct relationship between the two groups is indicated independently by the great similarity of the medusae of *Corymorpha* and *Pennaria* to the 'Sarsia' of *Syncoryne*.

A similar conclusion is reached from a study of the polymorphic tufts of Siphonanthus which have been termed 'cormidia'. The linear strings of these in *Macrostelia* are clearly secondary modifications, due to the excess of longitudinal over transverse growth (§ 10), while their more or less radial distribution in *Brachystelia* assimilates them to the reproductive branches of an ordinary arborescent Hydroid. Omitting Calycophora, in which important points are still uncertain, and in which simplifications of structure have evidently played a great part (§ 9), the typical and minimum constitution of a cormidium is a combination of polyp, gonopalpon (= blastostyle), and gonophores. This trimorphic combination is exactly comparable with the branch of a *Dicoryne* colony, consisting of a terminal polyp, a subterminal blastostyle, and its gonophores. There may be an extra polyp or blastostyle on the branch, as there may be extra polyps and some palpons in the cormidium, but the combination stated is essential and constant. In both cases the polyps themselves are sterile. This condition of pronounced trimorphism is adumbrated in *Disconanthus*, but is not found in *Tubularia*, or in any of the colonies, like *Pennaria*, which possess the Tubularian type of hydranth, with gonophores above



an aboral circlet of tentacles. All consist simply of polyps and gonophores. Thus, as in the case of the float, an origin from Disconanths is possible, but it is equally possible from Hydroids themselves, of a type less elaborate in structure than the Tubularians.

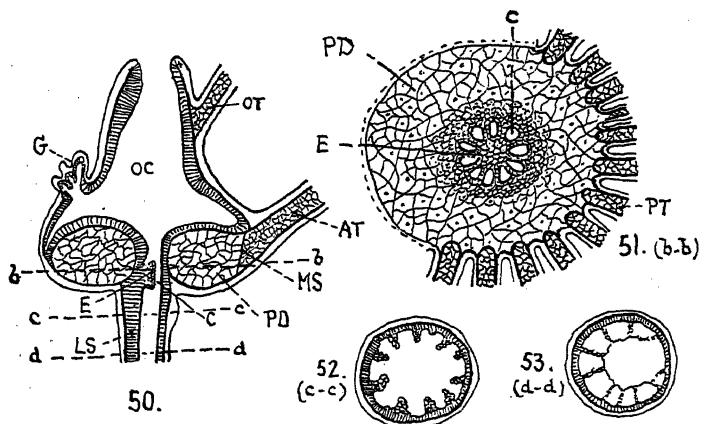
From these various considerations it seems to follow that, although the gap between Disconanths and Siphonanthus has been substantially reduced, the possibility of a diphyletic origin of Siphonophores has not been entirely excluded. The sharpest distinction that remains is that of their symmetries—radial budding and growth in one group, one-sided (ventral) budding and growth in the other; but, as a similar contrast separates Branchiocerianthus from Corymorpha without affecting their relationship, the separation at their origin may be no wider than that between one genus of a family and another. I have already indicated in my earlier chapters various points in Siphonophores themselves that can only be cleared up by new investigations, but for the settlement of this problem what we most need is more information, or more precise information, on the development and anatomy of certain Hydroids on which the solution of the problem of affiliation seems to depend. These requirements seem to be mainly as follows:

(a) the structure and development of the larvae of Corymorpha, Hybocodon, Pelagohydra, & Coryne Van Benedeni, Hincks (1868, fig. 4) (= pusilla of Van Beneden).

(b) the presence or absence of gastric diaphragm and cauline endochord and plexus in Acaulis and Hypolytus, and their mode of development in Corymorpha and Tubularia indivisa.

(c) the anatomy and complete development of Myriothela, with special reference to possible traces of diaphragm, endochord and plexus; gastrulation, and any evidences of a ventral non-muscular line and of bilateral symmetry; fixation of larva by sucker or tentacles. [On this, see Postscript, p. 193.]

My reasons for specifying so many non-colonial forms will appear from the following considerations. The specialization which attends the integration of bud-communities leads quickly



TEXT-FIGS. 50-3.

*Tubularia indivisa*. Sections after Grönberg, 1898, showing deviations from Corymorphine structure. *AT*, aboral tentacle; *G*, one of the short canals; *E*, endochord; *G*, gonophores; *LS*, longitudinal septa of stalk; *MS*, Mesogloea septum; *OC*, oral chamber; *OT*, oral tentacles; *PD*, parenchyma of diaphragm; *PT*, parenchyma of tentacles; *SP*, sieve-plate formed by remnants of the longitudinal canals opening into the single hydranth cavity.

Fig. 50.—Vertical section of hydranth, showing fusion of diaphragm with basal wall (N.B. Mesogloea between parenchyma of diaphragm and aboral tentacles); obliteration of aboral chamber; and reduction of endochord and stalk-canals.

Figs. 51, 52, and 53.—Transverse sections through *bb*, *cc*, and *dd*.

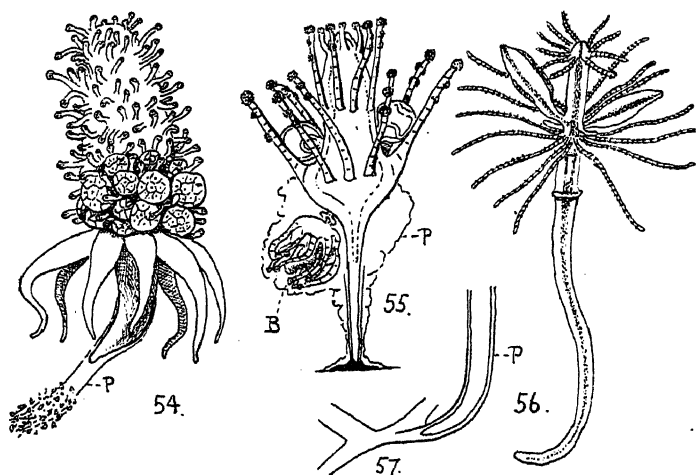
to the obliteration of earlier characters if they are not essential for the tasks of the various buds. Organisms, therefore, which have dropped out of the colonial race, at different levels, may reveal more completely some of the stepping stones of their advance. We are apt to think of Tubularians as the climax of Gymnoblaster evolution, and, so far as hydranth structure is concerned, that is manifestly true. But there is good reason to regard the structure of both hydranth and stalk as built up by a succession of definite additions and subtractions. The original position of Hydroid gonophores, as of polyps, was admittedly on a creeping stolon, and stages in their subsequent ascent up the side-walls of the polyps are seen in such simple forms as *Clavatella*, *Podocoryne*, and *Clava*, in the last of which they reach the zone immediately below the tentacles. In *Coryne* they just invade the tentaculate zone, and in the *Corymorphines* and *Tubularians* they pass on to the oral face of the hydranth, encircled by an equatorial whorl of specialized tentacles, unfortunately named 'aboral', but probably the lowest of the oral series (cf. *Tiarella*, Text-fig. 55).

Now in *Pelagohydra* both endochordal axis and peripheral plexus are enormously developed, thereby constituting its 'float', but the hydranth is perfectly simple with diffuse homogeneous tentacles, as in *Clava* or *Coryne*, and the gonophores are limited to the float, which clearly represents the Tubularian stalk. It is thus impossible to imagine the sessile forebears of *Pelagohydra* as solitary *Tubularias* or *Corymorphas*, owing to the basal position of their gonophores and their simple heads. There must have been—and may still be—a tribe of tall, simple, naked, polyps rising from a creeping stolon, with gonophores on their basal stalks, supported only by an endochordal axis; and this tribe was presumably ancestral not only to *Pelagohydra*, but to all the *Tubularians* with a similar internal axis. The presumed existence of such a tribe cannot be dismissed as mere phantasy, for in several other primitive Gymnoblasters (*Gemellaria* and *Clavatella*), with tall and slender naked stems, the hydranth is known to be supported by a corresponding differentiation of the endoderm of the stem, the narrow axial cavity being sur-

rounded by two specialized layers of an endodermal parenchyma (Allman 1871, Pl. vii, 5, and xviii, 11). After *Pelagohydra* broke away, the gonophores must have continued to climb, and eventually entered the tentaculate zone, thus completing the handsome but heavier structure of the Tubularian head. Apparently to meet this additional strain, the perisarc of the basal cups then extended upwards over the stalks, and the Tubularian character was fully attained.

But evolution clearly has not halted there. The simple unbranched stalks of the great *Tubularia indivisa* have been followed by the sparsely branched stalks of the smaller *Tubularia larynx*, and these by the full arborescent branching of the monopodial *Pennaria*—the sequence being also one of increasing number and diminishing size of the individual polyps. In the latter genus the composite structure of the Tubularian head has been fully retained, but the original endochordal axis has been completely replaced by the firm, light, perisarcal tubing of other forms, almost exactly as a cartilaginous tube first stiffened, and then replaced, the notochordal axis of early vertebrates.

This view of the evolutionary sequence derives strong support from Grönberg's investigations of the structure of the Tubularian stalk. In his Ray Society Monograph (1871) Allman attributed to *Tubularia* the same structure of the stalk as in *Corymorpha*, and figured a cross section showing an axial endochord surrounded by a ring of peripheral canals. But Grönberg, using the method of serial sections, has shown (1898) that this condition prevails only in the colonies with large unbranched polyps (*Tubularia indivisa*), and in them only through a very short region at the apex of the stalk (Text-figs. 50-3)—so short that in effect the endochord and canals together constitute merely a kind of sieve-plate between the cavities of hydranth and stalk. Below this level the endochord disappears, and only the side-walls of the canals persist for a short distance as a series of low free septa. These quickly degenerate into mere ridges, one cell thick, the edges of which are connected with those of their neighbours except for one or two irregular gaps. Farther down the ridges gradually disappear. In the smaller, but more arborescent, species (*Tubularia larynx* and *coronata*)



TEXT-FIGS. 54-7.

Various Monogastric Hydroids with intertentacular gonophores. *B*, bud ready for detachment; *P*, perisarc.

Fig. 54.—*Acaulis primarius*, Stimps. After G. O. Sars (1873).

Fig. 55.—*Tiarella singularis*. After F. E. Schulze (1876).

Fig. 56.—*Hypolytus peregrinus*. After Murbach (1899).

Fig. 57.—The same, showing the cauline extremity in its tube.

there are no canals at all—merely an occasional septum or cross-bar ('Bälkchen') which divides the lumen of the stalk with all the irregularity of vestigial structures.

Thus, as Grönberg correctly argued, *Tubularia* cannot be regarded as ancestral to *Corymorpha*, but the latter, in the structure of its stalk, indicates the ancestral type from which the *Tubularia* series has been derived, the new features being the perisarcal tubing and the atrophy of the original endochordal axis. Moreover, these characters lead directly to *Pennaria* which, with apparently no trace of endochord or plexus, has undergone further colonial integration by discarding the last relics of the creeping stolon, and carrying all its buds as outgrowths of the primary polyp body.

Without *Corymorpha* the structure of *Tubularia* would be unintelligible, and no one would suspect the secondary simplification which has led to *Pennaria*. But it does not follow, as Grönberg appears to have believed, that the ancestors of *Tubularia* were necessarily non-colonial, like *Corymorpha*. Many reasons, as well as the examples cited above, render it probable that *Corymorpha* was itself derived from a primitive polyp-community differing from that of a *Gemellaria* or *Clavatella* merely in details. Very little is known of the development of any *Corymorphine*. E. T. Browne's promised drawings of *Amphicodon* (*Hybocodon*?) seem never to have been published (Browne, 1895).

Of the other monogastric Hydroids, *Acaulis* (Text-fig. 54) is probably a *Corymorphine* which has developed a long prehensile proboscis on the lines of a *Myriothele*, just as various *Myriothele* lines of deep water show remarkable modifications for limicolous or arenicolous existence on *Corymorphine* lines (see Bonnevie, 1898). Sars obtained his specimens from muddy ground off Lofoten in 40–100 fathoms. It would appear to live immersed to the level of its large extensile aboral tentacles, which doubtless radiate over the surface and guide its proboscis to approaching prey. Attachment to the mud appears to be no longer by rootlets but by the adhesiveness of its loose perisarcal sheath. In view of its diminished stalk, a knowledge of its internal structure would be of much value.

*Hypolytus* (Text-fig. 56) is found in the eel-pond at Woods Hole, adhering to blades of *Zostera* by the whole length of its perisarcal tube, which can be deserted and renewed. It has obvious affinities with *Corymorpha* and *Tubularia*, but its tentacles have a more primitive aspect, and only additional knowledge of its internal structure can settle its history. Judging from Murbach's figures, which include no sections, it would seem that endochord and plexus have disappeared even more completely than in *Tubularia*, but the diaphragm may be represented by the 'deeply pigmented band' below the tentacles, for which Murbach 'found no adequate explanation'.

*Tiarella* (Text-fig. 55) is an obscure little Hydroid, interesting from its production of free buds like *Myriothele*, which F. E. Schulze found for a few weeks one year attached to *Cystosira* drifting about the Bay of Muggia near Trieste. He referred it with considerable hesitation to the Pennariidae, but it has three equidistant whorls of tentacles, all capitate, so that Corynoid affinities are equally possible, and its perisarcal sheath is essentially Corymorphoid.

After this lengthy discussion the evolutionary situation may be summed up in few words. There are indications of a Myriothele relationship of Siphonanthus which needs to be reconciled with the Corymorphine affinities of Disconanthus before a common origin can be firmly assured. Pelagohydra, Siphonanthus, and Disconanthus may in fact be independent pelagic offshoots from three different types of Gymnoblasic Hydroid, the larvae of which failed to attach themselves but succeeded in keeping afloat until their gonophores ripened. Pelagohydra absolutely, and Disconanthus to some extent, may be said to have survived by the adaptive aggrandisement of their oozoids, at the expense of their colonies, while Siphonanthus, by precocious budding, elaborated the colony at the expense of the oozoid. The latter tribe somehow acquired a new feature, their one-sided mode of gastrulation, which predetermined a bilateral symmetry. Growth in width at first compelled a whorled arrangement of their buds, but the divergent forms it assumed suggest great instability (*Brachystelia*). Some of these then turned it to real use by suppressing all growth in

width for greatly increased length, and thus trailed a long line of prehensile buds behind the locomotive fore-body (*Macrostelia*). Evidently their free embryonic life, peculiar gastrulation, and precocious budding have been distinctive factors in *Siphonanth* evolution.

### 13. SYSTEMATIC.

From the systematic standpoint the following scheme sums up the more definite results of the present study. Its main feature is its reversal of the order in which Chun arranged the principal groups (1897). In detail I go back to Eschscholtz's (1829) tripartite division into *Chondrophorae* (Chamisso, 1821), *Physophorae*, and *Calycophorae* (Leuckart, 1854), which was broken up by Haeckel, but have combined Haeckel's *Disconanthae* and *Siphonanthae* with it to mark the major gap between the *Chondrophorae* and the remainder. All the feminine terminations, however, which originally marked the '*Siphonophorae*' and its subdivisions as '*Medusae*' (Chamisso) or '*Akalephae*' (Eschscholtz), have been changed to the simpler and more conventional neuter plurals.

Haeckel claimed his '*Physonectae*' to be synonymous with Eschscholtz's '*Physophorae*', but in fact he applied the term to a mere section of that comprehensive group, which is here revived under the nearest possible approach to the original designation, viz. *Physophorida*. As a title for one of its sections '*Physonectae*' is undesirable, both from its liability to confusion with the historic ordinal name and from its failure to convey any distinctive meaning in contrast to '*Cystonectae*'. I therefore propose to substitute the title '*Amphinecta*', which emphasizes the outstanding difference between the surviving sections. Both possess a similar unchambered float (i.e. they are *Physophorida*), but one lot swim with the bladder alone (*Cystonecta*), the other with both bladder and special appendages, i.e. bracts or necto-calyces (*Amphinecta*). In both these sections, pending the establishment of definite lineages, I have provisionally employed Haeckel's descriptive terms *Macrostelia* and *Brachystelia* in his sense.



## SIPHONOPHORA

## Floating bud-communities of Gymnoblastic Hydromedusae.

- I. DISCONANTHA.—Oozoid with a large chambered air-float and an aboral ring of tentacles (dactylozooids?). Secondary polyps (blastozooids) non-tentaculate, ranged around the oozoid with radial symmetry, and bearing Anthomedusan gonophores ultimately detached. No bracts, palpons, blastostyles (s.str.), or nectocalyces. Larva an Actinula with a mesogastric diaphragm (Conaria). = Order CHONDROPHORA: including *Porpita*, *Porpema*, *Velella*.
- II. SIPHONANTHA.—Colonies bilaterally symmetrical, the oozoid with a mesosomatic budding tract in the mid-ventral line, which divides the colony into a locomotive nectosome and a gastro-genital siphosome. Polymorphism to various degrees, but always with palpons, gonopalpons, or nectocalyces. Polyps each with a single basal tentacle. Gonophores usually sessile on blastostyles (gonopalpons), together forming the gonodendra. Polyps and gonodendra usually associated in pairs (polyp below, gonodendron above), forming 'cormidia' with or without the addition of bracts and palpons.
- A. PHYSOPHORIDA.—With unchambered float and ectodermal gas-gland. Oozoid either short and stout, bearing cormidia in whorls or spires (*Brachystelia*) or greatly attenuated, with cormidia more or less metameric (*Macrostelia*).
- (1) CYSTONECTA.—Float large with apical pore, usually with hypocystic villi. Nectosome without locomotive or other appendages. Stem without bracts.
- BRACHYSTELIA.—*Epibulia*. *Physalia*.
- MACROSTELIA.—*Rhizophysa*. *Bathypphysa*. *Pterophysa*.
- (2) AMPHINECTA (= *Physonectae*, Haeckel)—Float closed, usually small with pericystic septa; but large, with incomplete septa in *Anthophysa*.
- (a) BRACHYSTELIA.—*Anthophysa* and *Athorybia* (Nectosome with paddling bracts but no nectocalyces); *Nectalia* (with bracts and nectocalyces); *Physophoridae* and *Rhodaliidae* (with nectocalyces only).
- (b) MACROSTELIA.—*Agalmidae*, with bracts in larva, nectocalyces in adult, and numerous stem-bracts, e.g. *Agalma*, *Stephanomia* (= *Halistemma* and *Agalmopsis*); *Forskaliidae*; *Apolemidae*.
- B. CALYCOPHORIDA.—No float (aboral region of larva aborted), but a subapical position assumed by one, two, or more nectocalyces, usually with a somatocyst. All *Macrostelia*, the cormidia usually detached as free-swimming sub-colonies ('*Eudoxiae*') with an anterior covering bract enclosing phyllocyst with oil-globule.

Stephanophyes, with a corona of four nectocalyces and slender palpons alternating with sessile cormidia (connecting with Amphinecta?); Hippopodius, with a turret of nectocalyces and sessile cormidia; Galeolaria,<sup>1</sup> Praya, and Diphyes, with two nectocalyces; Muggiaea and Sphaeronectes, with one nectocalyx.

Although this classification is a mere statement of distinctive facts of structure, it suggests the course of Siphonophore evolution as a progress from passive flotation, through various combinations of flotation with active modes of locomotion, to a climax of purely muscular methods of swimming and colonial simplification in Calycephorida.

The Chondrophora and Brachystelia are essentially epipelagic, while the Macrostelia range through a great variety of depths, several Cystonects and Calycephores being definitely bathypelagic (cf. Bigelow, 1911; Bigelow and Sears, 1937).

<sup>1</sup> I must decline to substitute for this expressive name (galea, a helmet: cf. Text-fig. 5), so long imbedded in Siphonophore research, the barren and barbarous Sulculeolaria of Blainville, merely because some obscure Mollusc is also entitled to it. Who would ever confuse Galeolaria the Mollusc with Galeolaria the Siphonophore? Such useless applications of the law of priority severely handicap the progress of Zoology.

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## POSTSCRIPT, 21 March 1946.

Three important memoirs on *Myriothela* were overlooked in the writing of this paper in 1943-4, viz.:

- Benoit, P., 1925.—'Arch. Zool. Exp.', 64.  
 Manton, S. M., 1940.—'Rep. Brit. Graham Land Exp. 1, 4'.  
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Benoit throws reasonable doubts on the normality of Hardy's 'buds' (p. 145, supra), which were nevertheless seen by me at the time; and Manton, along with other things of interest, renders it almost certain that the larva attaches itself by a number of aboral ('hydrorhizal') tentacles, and not by a basal disc or 'sucker' (p. 146, supra).



# Further Observations on *Amoeba discoides*.

By

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With 3 Text-figures.

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## INTRODUCTION.

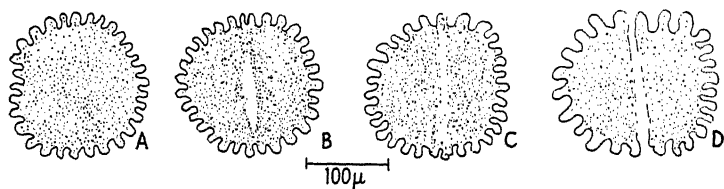
WHEN in 1938 I wrote 'An Account of *Amoeba discoides*, its Culture and Life History', I was aware of some lacunae in the work. These were due to lack of sufficient material at that date. Because the large, free-living amoebae grow so slowly it takes considerable time and constant care to accumulate strong, healthy, laboratory cultures from a very limited original source. During the intervening years, however, I have had excellent success in the laboratory cultivation of this amoeba and in consequence abundant material at every stage of the life-cycle to enable me to make deeper and more detailed studies of each stage. The observations recorded in the following pages are the results of these studies which complete the life-history of *Amoeba discoides*.

### 1. CYTOPLASMIC DIVISION.

When the amoeba is about to divide it withdraws all the long pseudopodia, fastens itself to the sub-stratum and becomes spherical, the sphere being covered all over with very short, blunt pseudopodia (Text-fig. 1 A).

Compared with the original size of the amoeba in its creeping or floating condition this sphere is small, which fact suggests that the cytoplasm has become condensed. The first sign of the actual division is the appearance of a clear area in the cytoplasm at about the middle of the sphere and at right angles to the equator (Text-fig. 1 B). Since sufficient depth of water must be allowed for division to take place normally and uninterruptedly the observations have to be carried out under

a no. 3 objective. With this magnification it is impossible to tell whether the clear area just referred to is a real break or only a clearing in the cytoplasm. Whatever its nature it spreads gradually northwards and southwards to the poles (Text-fig. 1 c); then as a general rule it closes up again and the amoeba sphere looks as if no division had taken place. After a minute



TEXT-FIG. 1.

Diagrammatic representation of fission in *Amoeba discoides*.

A, Spherical form assumed before division—numerous short, blunt pseudopodia. B, First appearance of the clear area in the cytoplasm. C, The clear area has spread to the poles. D, Actual division accomplished.

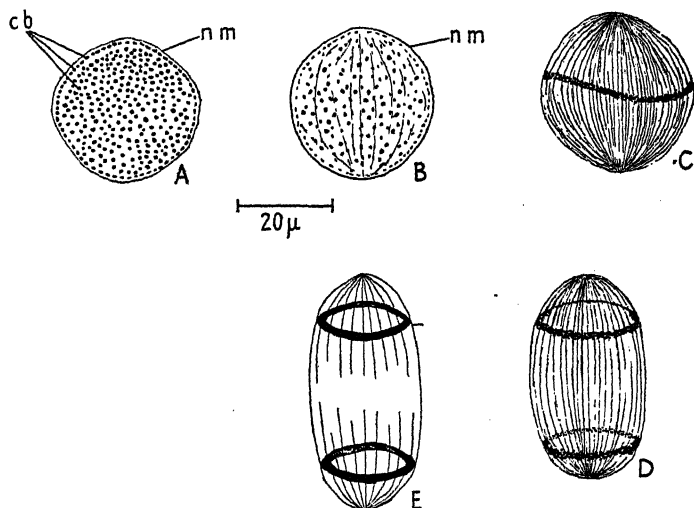
or two large pseudopodia are pushed out, the cytoplasm begins to flow rapidly and the two daughter amoebae move slowly away from each other, the actual dividing line being the originally observed clearing or break (Text-fig. 1 D). Almost at once the daughter amoebae look and are in actual linear measurement as large as the mother amoeba, suggesting that the cytoplasm is now very thinly spread out and has probably absorbed water, perhaps in the short time which elapses between the completion of the division and the moving apart of the individuals.

One division-sphere was seen to divide into three individuals. Ordinary division into two took place first; then, immediately after, one of the daughters again divided into two. This probably often occurs in *Amoeba discoides* since in fixed and stained individuals two or three large nuclei are quite common. I have also seen multiple mitosis in stained preparations.

## 2. MITOSIS.

When division is about to take place the nucleus loses its staining capacity, the nuclear membrane becomes exceedingly

thin, and the karyosome disappears. The chromatin masses are now evenly distributed (Text-fig. 2 A); but they are small, and because of their inability to stain it is exceedingly difficult to discover the nucleus in the stained amoeba. This phase may be looked upon as early prophase. The next stage (Text-fig. 2 B),



TEXT-FIG. 2.

Mitosis in *Amoeba discoides*. *nm*, nuclear membrane; *cb*, chromatin blocks. A, Early prophase: membrane thin, chromatin blocks evenly distributed, karyosome has disappeared. B, Late prophase: spindle fibres condensing out. C, Metaphase: all the very small numerous chromosomes round the equator of the nucleus. D, Late anaphase: polar caps dome-shaped. E, Telophase: chromosomes fused into a band. (Drawing more or less diagrammatic.)

although still more difficult to detect, shows indications of a late prophase in which the achromatic spindle threads begin to appear. In metaphase (Text-fig. 2 c) the very small chromosomes are arranged on the equator of a more or less barrel-shaped nucleus. The nuclear membrane persists and for the first time the chromosomes are easily visible though they are too small and too numerous to be counted. There are no visible centrosomes and no asters. In anaphase the two daughter



sets of chromosomes travel to the poles (Text-fig. 2 D). The spindle poles are dome-shaped, and in this respect *Amoeba discoides* differs from *Amoeba lescherae* (Taylor and Hayes, 1944) and from *Amoeba proteus*, where they are conical (Dawson et al., 1937).

In telophase (Text-fig. 2 E) the chromosomes sometimes coalesce and appear as a deep-staining band around the circumference of the base of each dome.

The initiation of division of the cytoplasm causes the two daughter telophasic-nuclei to be carried to opposite ends of the 'fission-amoebea' where the daughter nuclei are reconstructed into the resting stages.

### 3. THE EMISSION OF CHROMIDIA, PREPARATORY TO CYST FORMATION.

On p. 474 of my paper (1938) I stated that I had not seen chromatin blocks actually escaping from the nucleus of *Amoeba discoides*. Since then I have often seen chromidia, i.e. chromatin blocks, just outside the nucleus and scattered through the cytoplasm of many fixed and stained adult *Amoeba discoides*. These preparations had always been made by the usual long and laborious method of fixation, dehydration, clearing, &c.

My thanks are due to Dr. Pontecorvo for calling my attention to the acetic-alcohol plus aceto-carmin technique; for, by its use, large numbers of adult amoebae considered due to undergo cyst formation can rapidly be examined.

After 24 hours in acetic-alcohol the amoebae can be examined at once in aceto-carmin when the escaping chromidia can be detected quite easily in those individuals where the phenomenon is taking place (Taylor and Hayes, 1944).

### 4. EXCYSTATION AND EARLY DEVELOPMENT OF AMOEBA DISCOIDES.

#### Method of Obtaining Cysts.

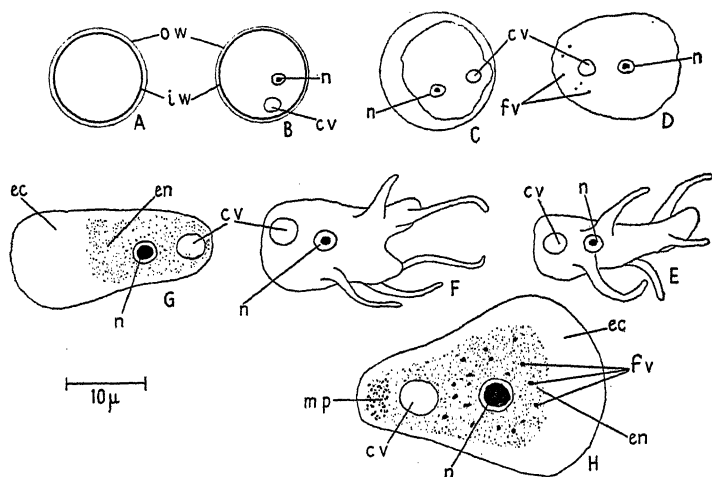
In December 1942, while examining a Petri-dish culture which had been in a flourishing condition for several months,

I noticed that most of the individual amoebae were packed with very large nutritive spheres and I suspected that these individuals were ready to form cysts (Taylor and Hayes, 1944). Consequently several slides were prepared by placing on each five or six of these large amoebae. The preparations were then placed in a damp chamber and left for some weeks, care being taken to add a little water when necessary. When the preparations were examined it was found that most of the amoebae had disintegrated, giving rise to cysts, excysting amoebulae, and early stages in the development of the excysted amoebulae. The amoebae not removed from the original culture gradually disappeared and an examination of the bottom of the Petri-dish revealed cysts and developmental stages similar to those found on the slides.

#### Description of Cysts and of Young Encysted Amoebae.

The living cysts of *Amoeba discoides* are spherical with a diameter varying from  $9\ \mu$  to  $12\ \mu$ . They are provided with a very thin outer wall and a much thicker inner one (Text-fig. 3 A). The inner thickened wall appears to be the outer surface of the encysting amoeba, for when excystation is completed the outer thin wall alone remains behind. The interior of the cyst consists of highly concentrated, viscid, non-granular cytoplasm in which the newly differentiated nucleus is the first definite structure to be seen. Soon after this a small contractile vacuole begins to pulsate at regular intervals (Text-fig. 3 B). The inner cyst wall now becomes permeable, that is, it is gradually becoming an integral part of the central mass of cytoplasm. The fact that the contractile vacuole is able to function indicates that there must be some communication between the interior of the cyst and the surrounding water, although no visible break in the outer wall can be detected (Taylor and Hayes, 1944). Later the cytoplasm becomes capable of amoeboid movement, contracting slightly from the cyst wall (Text-fig. 3 C). It is less viscid and is now ready to emerge from the cyst. The newly emerged amoebula, though of a fluid-like consistency and of extreme transparency, moves

but little at first and these slight movements are of the nature of little convulsions. It does, however, engulf a few very small food particles during this period (Text-fig. 3 D). It next tends to float, numerous pseudopodia are pushed out and a few



TEXT-FIG. 3.

A, Cyst of *Amoeba discoides*. B, Differentiation begins in cyst. Nucleus distinguishable. C, Preparation for hatching—inner wall of cyst 'dissolved'. Contractile vacuole functioning. D, Newly hatched amoebula, ingestion of food. E, Amoebula begins to move actively by means of long pseudopodia. F, Later stage. G, Amoebula assumes the 'limax' stage . . . ectoplasm and endoplasm clearly differentiated. H, Older amoeba, metabolic products as well as food-vacuoles. *cv*, contractile vacuole; *ec*, ectoplasm; *en*, endoplasm; *fv*, food vacuole; *iw*, inner wall; *mp*, metabolic products; *n*, nucleus; *ow*, outer wall. All drawings made from living material.

more particles are engulfed. The cytoplasm though becoming gradually coarser is so thin that it is exceedingly difficult to distinguish ectoplasm from endoplasm (Text-fig. 3 E to F).

The next stage in development may be called the 'limax' stage as all the pseudopodia are withdrawn and the amoebula begins to creep about in limax fashion. The cytoplasm, colourless and very fluid, moves easily and rapidly. The ectoplasm

has become fully differentiated and is voluminous and very transparent; the endoplasm is by now quite granular. Numerous food particles are always present. The nucleus is conspicuous and large in comparison with the size of the amoeba. By contrast with that of *Amoeba lescherae* the karyosome is large.

From now onwards growth manifests itself by increase in bulk rather than increase in length. The endoplasm shows metabolic products in addition to food vacuoles (Text-fig. 3 H): the ectoplasm is less voluminous. Periodically, as in the adult, the actively moving amoebae assume a resting stage during which they are more or less spherical with numerous blunt pseudopodia, but from this condition they easily pass again into the 'limax' state.

Further stages in development have already been described in my former paper (1938) on *Amoeba discoides*. I had not at that time seen the excystation and early stages described above.

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Taylor, Monica, and Hayes, C., 1944.—*Ibid.*, 84, 295.



# Cytochemical Differentiation between the Pentose and Desoxypentose Nucleic Acids in Tissue Sections

By  
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With Plate 7

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ANIMAL tissues contain two types of nucleic acid: the desoxypentose, or thymo-, nucleic acids whose distribution is confined to the nucleus; and the pentose, or yeast, nucleic acids which can occur in both nucleus and cytoplasm. For many years it has been possible to identify desoxypentose nucleic acid by means of the Feulgen reaction, although, as has been frequently pointed out (Lison, 1936; Stowell, 1943), this must be carried out under carefully standardized and controlled conditions if its results are to be reliable. No corresponding test for pentose nucleic acids existed until Brachet (1940, 1941) showed that it was possible to identify pentose nucleic acids by comparing sections stained with basic dyes with other sections similarly stained following treatment with a pancreatic extract containing the enzyme ribonuclease. Therefore a distinction between the two types of nucleic acid may be made by applying both the Feulgen and Brachet tests to the same material. However, since the stains used in the two cases are different, and one set of sections is subjected to acid hydrolysis and the other is not, it may be difficult to relate the two cytological appearances to one another.

The present paper describes a simple differential test for the two types of nucleic acid involving the use of highly purified enzyme preparations. It differs from Brachet's (1940) test in two particulars: (1) all sections are stained with the same set of dyes after incubation in ribonuclease, desoxyribonuclease, or plain buffer solution; so that comparisons between the differently

treated sections are more easily made; (2) an attempt has been made to make the test more reliable by the use of highly purified enzymes, with precautions to restrict their action to a single substrate.

The test itself is performed as follows: Tissues are fixed by perfusion with Carnoy, Zenker, or chilled acetone,<sup>1</sup> dehydrated, and embedded by the usual methods. After Bouin or formalin fixation the method gives less clear-cut results. Blocks are cut at  $10\mu$ , and adjacent sections attached to each of four slides, which are dewaxed in chloroform, rehydrated, and washed in three changes of distilled water. One slide is then incubated for 1 hour at  $56^{\circ}\text{C}$ . in M/15 acetate-veronal buffer of pH 6.75 containing 1 mg. per 100 c.c. of crystalline ribonuclease, prepared according to Kunitz (1940). Since it has been suggested that even crystalline ribonuclease has some residual proteolytic activity (Cohen, 1945), due presumably to adsorbed impurity, this is destroyed by heating the enzyme solution to  $80^{\circ}\text{C}$ . for 10 minutes before use. A second slide is incubated for 1 hour at  $37^{\circ}\text{C}$ . in an M/40 acetate-veronal buffer of pH 7.5 containing 1 mg. per 100 c.c. highly purified pancreatic desoxyribonuclease, prepared as described by McCarty (1946). Since this enzyme is heat-labile, the solution was not heated to remove any possible residual proteolytic activity. It was found, however, as also noted by McCarty (1946), that at the dilution used proteolysis is negligible during one hour's incubation. The enzyme solution also contained gelatine at a concentration of 0.01 per cent. as protective colloid, and also 0.003M  $\text{MgSO}_4$ , the magnesium ions being necessary to activate the enzyme. The remaining two slides are used as controls, one being incubated in each of the buffers made up exactly as described above, only without the added enzyme. After treatment the four slides are washed in several changes of distilled water and then passed together through the following two solutions: (1) 0.50 per cent. celestine blue in acid solution as described by Lendrum (1935); duration

<sup>1</sup> Acetone fixation was investigated as it allows the performance, upon adjacent sections, of the reactions for alkaline and acid phosphatase described by Gomori (1941) and Wolf, Kabat, and Newman (1943), whereby nucleic acid distribution and phosphatase activity may be correlated.

of staining—5–15 minutes followed by 10 minutes washing in running tap-water; (2) 0.25 per cent. Pyronin G (pyronin 0.25 gm.; 96 per cent. alcohol 2.5 c.c.; glycerin 20 c.c.; phenol 0.5 gm.; water to make 100 c.c.); duration of staining 30 minutes. Following this the slides are rinsed in tap-water, blotted dry with filter paper, and transferred direct to a mixture of three volumes of xylene with one of absolute alcohol. This mixture, originally suggested by Unna, dehydrates the sections without extracting much of the pyronin; celestine blue is not extracted by alcohol. Finally, the slides are cleared in xylene and mounted in neutral Canada balsam.

On slides so treated the differential distribution of desoxypentose and pentose nucleic acids may be ascertained by comparing the two enzyme-treated slides with the two controls. For example, fig. 1, Pl. 7, is a photomicrograph of a control section of the cerebellum of a guinea-pig, fixed in acetone and stained in celestine blue–pyronin. The nuclei of the granular layer stain intensely with celestine blue, while the Purkinje cell layer is strongly coloured by pyronin. Fig. 2, Pl. 7, shows the section treated with desoxyribonuclease. The Purkinje cells still have a strong affinity for pyronin, while the nuclei of the granular layer are hardly stained, and then by pyronin rather than celestine blue. In fig. 3, Pl. 7, the section treated with ribonuclease is shown. The nuclei of the granular layer take the celestine blue strongly, while the Purkinje cells are pale and hard to differentiate from the background. From this we may conclude that the Purkinje cells are rich in pentose nucleic acid, but relatively poor in desoxypentose nucleic acid, while there is a considerable concentration of the latter in the nuclei of the granular layer immediately below them. Figs. 4, 5, and 6, Pl. 7, show that the test may be used to differentiate the two acids within the cells themselves. Fig. 4, Pl. 7, shows a Purkinje cell of a type very common in the cerebellum. The nucleolus stains strongly with pyronin, while there is a dense cap of pyronin-stained substance on the nuclear membrane and a diffuse granular zone of pyronin staining in the cytoplasm. The nucleus also contains matter staining with celestine blue. In fig. 5, Pl. 7, a similar cell is shown, this time after



treatment with desoxyribonuclease. The dense pyronin-stained nucleolus, nuclear membrane, and cytoplasm remain, but the blue staining masses in the nucleus are absent. A third cell, stained after incubation with ribonuclease is shown in fig. 6, Pl. 7. Here the cytoplasmic granules and the cap on the nuclear membrane staining with pyronin have been removed, but the nucleolus is still visible staining faintly with celestine blue rather than pyronin, as do the other masses within the nucleus. From this test it may be concluded that cells of this type contain both types of nucleic acid, distributed in the following way: (1) pentose nucleic acid; there is a large amount of this acid in the cytoplasm, in addition to especial concentrations at the nuclear membrane and in the nucleolus; (2) desoxy-pentose nucleic acid; this is confined to the nucleus, there being a small amount in the nucleolus, while the rest is present elsewhere within the nucleus.

Tests with enzymes of the type described above have recently been severely criticized by Danielli (1946) on two main grounds: firstly, that enzyme preparations, however highly purified, are rarely specific for one substrate only; secondly, that removal of part of a cell by nucleases does not prove this part of the cell to be composed mainly of nucleic acid, since there may be only a small amount of nucleic acid present in the key position of a matrix binding two masses of protein material together; also nucleic acids within the cell may be protected from the action of enzymes by a shell of protein. In the above test an attempt has been made to guard against the first of these objections by precautions designed to ensure the specificity of the enzymes used. The important point is that while both enzymes may have slight proteolytic activity, which can be rendered ineffective by heating in the one case and dilution in the other, each enzyme only attacks one of the two types of nucleic acid, and has been shown to be entirely without effect upon the other (Kunitz, 1940; McCarty, 1946). Figs. 7 and 8, Pl. 7, show the specificity of the McCarty enzyme. Fig. 7 shows the granular layer of the guinea-pig cerebellum, stained by the Feulgen technique. Fig. 8 shows an adjacent section, stained in Feulgen after incubation in buffer and desoxyribonuclease. As regards the second objection,

it must be remembered that the above test is performed upon sections of fixed tissues. Under these circumstances it must be expected that the proteins have been made insoluble. Removal of nucleic acid acting as a matrix between protein masses need not therefore cause one protein moiety to go into solution. It is far more likely that the nucleic acid itself will be removed and the protein left as part of the section attached to the slide. Moreover, it is unlikely that protective protein shells, particularly as monolayers, will exist in fixed tissues. Did they exist, they would prevent staining by dyes as much as enzyme action.

It must be borne in mind, however, that difficulty may be experienced in relating the distribution of nucleic acids as revealed by the above test to the living cell. This is, however, a difficulty which attends the interpretation of all images seen in fixed and stained sections. In addition, it must be emphasized that a negative reaction to the above test does not indicate that either type of nucleic acid is absent from the section investigated; the substance may be present, but in such a physical or chemical state that it does not react with the enzyme used. What the test does is to enable us to distinguish the two types of nucleic acid when they are present. With these reservations, it is claimed that the above test provides a simple means of differentiating pentose from desoxypentose nucleic acid in sections of fixed tissue.

The author wishes to thank Professor R. A. Peters for permission to work in the Dept. of Biochemistry at Oxford when preparing the enzymes used in this work.

*Addendum.* Since this paper went to press, Catcheside and Holmes (personal communication) have reported using McCarty's (1946) pancreatic desoxyribonuclease to remove desoxypentose nucleic acid from chromosomes. They also found the enzyme to be almost without proteolytic action.

## EXPLANATION OF PLATE

All figures are untouched photomicrographs of sections stained, except where otherwise stated, by the celestine blue-pyronin technique.

Fig. 1.—Longitudinal section through a convolution of the cerebellum of the guinea-pig, after 1 hour's incubation in M/40 acetate-veronal buffer at pH 7.5. Note: granular layer of nuclei; Purkinje cell layer; white matter.

Fig. 2.—Adjacent section to fig. 1, after 1 hour's incubation in M/40 acetate-veronal buffer pH 7.5 containing 1 mg. per 100 c.c. highly purified desoxyribonuclease.

Fig. 3.—Adjacent section to fig. 1, after 1 hour's incubation in M/15 acetate-veronal buffer pH 6.75 containing 1 mg. crystalline ribonuclease per 100 c.c.

Fig. 4.—Single Purkinje cell from the cerebellum of the guinea-pig after 1 hour's incubation in M/15 acetate-veronal buffer pH 6.75.

Fig. 5.—Single Purkinje cell from the cerebellum of the guinea-pig after 1 hour's incubation in M/40 acetate-veronal buffer pH 7.5, containing 1 mg. per 100 c.c. highly purified desoxyribonuclease.

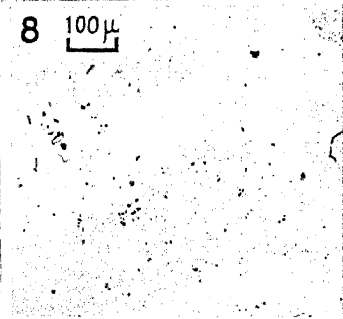
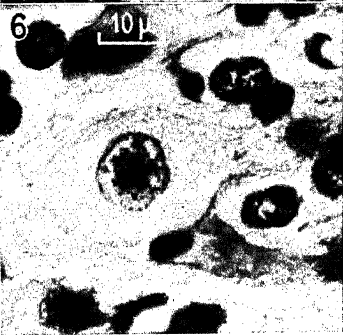
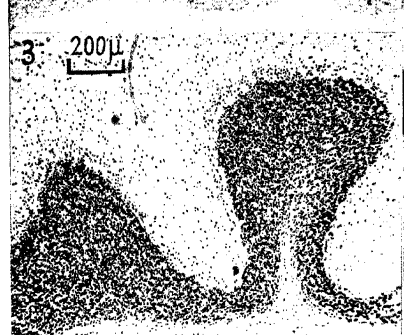
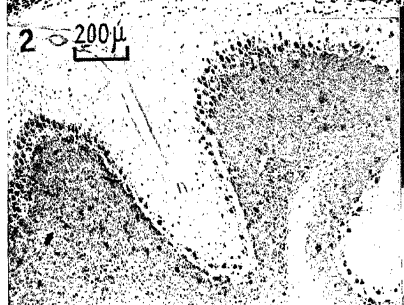
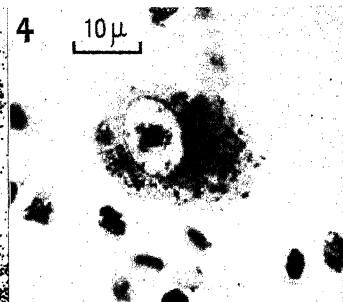
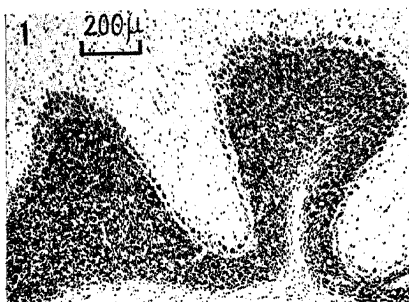
Fig. 6.—Single Purkinje cell from the cerebellum of the guinea-pig after 1 hour's incubation in M/15 acetate-veronal buffer pH 6.75, containing 1 mg. per 100 c.c. crystalline ribonuclease.

Fig. 7.—Transverse section of a convolution of the cerebellum of the rabbit, after 1 hour's incubation in M/40 acetate-veronal buffer pH 7.5. Feulgen reaction.

Fig. 8.—Adjacent section to fig. 7, after 1 hour's incubation in M/40 acetate-veronal buffer pH 7.5, containing 1 mgm. per 100 c.c. highly purified desoxyribonuclease. Feulgen reaction.

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# The Tegumental Glands in the Land Isopoda

## A. The Rosette Glands

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With 11 Text-figures

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### I. INTRODUCTION.

THE tegumental glands of the Decapod Crustacea have been described by many workers, but in the case of the Isopoda the corresponding glands have been much less fully investigated. The literature consists very largely of a rather confused mass of unrelated facts, and none of the published accounts gives a complete survey of all that is known. In many cases the author is concerned only with one particular type of gland, or with the glands of one particular region of the body. Often he fails to distinguish between different types of gland, and frequently he has not been fully acquainted with previous work.

In the Decapoda the tegumental glands consist of 'small, rosette shaped organs which are distributed everywhere beneath the surface of the integument, in the foregut and hindgut and in the gill chamber as well as over the entire surface of the body' (Yonge, 1932). In the terrestrial Isopoda or woodlice they are remarkable for their abundance, ubiquity, and variety. They are found in almost every part of the body, sometimes almost completely filling the structure in which they lie, and, unlike those of the Decapoda, they are of several different kinds.

In the terrestrial Isopoda tegumental glands may be present in the head, thorax, and abdomen; lateral plates and telson; antennae, mouth-parts, legs, gills, and uropods. Structurally they fall into at least five groups, and if, as some investigators have suggested, 'Zenker's Organ' in *Asellus* is to be regarded as a group of modified tegumental glands, then the Isopoda possess no less than six distinct varieties of tegumental gland. Only one of these is known to occur outside the group Isopoda, and only two, apart from 'Zenker's Organ', have been described before. The glands of three of the six types are unicellular (or rather in one case acellular), and of the other three compound.

They may be classified as follows:

#### A. Rosette Glands.

These are confined to the head and mouth-parts, and in essentials resemble those of the Decapoda.

#### B. Lobed Glands.

These glands are often greatly developed in the uropods and lateral plates and may be present in or near the gills. They have been variously named, usually in accordance with their position in the body, or, as in the case of 'Weber's Glands', after the investigator who first described them (Weber, 1881). They appear, however, to have precisely the same structure in all parts of the body, and since in their lobed structure they possess a striking and unique characteristic, it is proposed to call them 'Lobed Glands'. They are totally unlike any glands described in other groups of Crustacea.

### C. Compound Glands in Antennae.

A group of three elongated, compound glands is often present in the long (fourth) segment of the antenna. They are somewhat similar in appearance to glands which are found in the legs of certain Amphipoda and which secrete cement for building nests (Nebeski, 1880). They cannot be called 'Antennal Glands' as that name has already been used for certain excretory organs sometimes present in the antennae of the Isopoda and other groups. They are quite distinct from these.

### D. Small Compound Glands.

These consist of small groups of cells scattered throughout the body, and particularly numerous in the legs and antennae.

### E. Large Unicellular Glands.

Groups of large gland cells are found near the anterior edge of the lateral plates in the thorax.

### F. 'Zenker's Organ'.

This structure is confined to the genus *Asellus*, and consists of a number of extraordinarily large cells on each side of the intestine. These cells may be homologous with the lobed glands.

Of these structures, only the lobed glands and, to a less extent, the rosette glands and 'Zenker's Organ', have been previously described. Owing to their unusual appearance and their relatively enormous dimensions, and to the fact that their secretion is clearly visible to the naked eye, the lobed glands have attracted the attention of a considerable number of workers, and various functions have been assigned to them. A few investigators have studied the rosette glands in the Isopoda, but there is no complete account of their distribution and structure. Also, since the appearance of the most recent account, it has been shown (Yonge, 1932) that in the Decapoda the rosette glands are concerned with the formation of cuticle, and it appeared desirable to investigate the possibility of their having the same function in the Isopoda. The compound glands in the antennae have not, as far as is known, been described before.



This is believed to be true also of the small compound and large unicellular glands. They certainly do not appear to have been described in detail. The present paper deals exclusively with the rosette glands. Accounts of the other types of gland will appear in due course.

This work was carried out in the Department of Zoology at the University of Bristol, at the suggestion and under the supervision of Professor C. M. Yonge, whose unfailing help and encouragement it is a pleasure to acknowledge.

## II. MATERIAL AND METHODS.

The glands were studied from the point of view both of structure and of function. Their structure was determined by examination of transverse and longitudinal sections of *Porcellio scaber* Latr., since in this species all types of gland (except 'Zenker's Organ') are well developed. Collinge (1921) states that 'it has frequently been pointed out by investigators upon the minute anatomy of the Terrestrial Isopoda, how numerous are the difficulties that are presented in connection with the preservation and preparation of the different organs for histological investigation'. In the present work, a variety of fixatives (including Bouin's, Flemming's and Zenker's fluids, Dubosq-Brazil and Zenker-Formol) was used, but in every case the results were poor. It was eventually realized that this was at least partly due to a reaction between the acid present in the fixative and the calcium carbonate impregnating the integument. This reaction results in the formation of a considerable volume of carbon dioxide, and it is apparently this evolution of gas (bubbles of which accumulate in the alimentary canal) that causes distortion of the tissues. This difficulty was overcome by fixing specimens in neutral or alkaline solutions, and slowly decalcifying afterwards. Corrosive sublimate and corrosive formol gave better fixation. Excellent results, however, were obtained by fixing in 95 per cent. alcohol, both cold (room temperature) and hot (60° C.), and this reagent was subsequently used in the preparation of all sections, except where particular staining methods required special fixation. Specimens were then embedded in paraffin wax, and

microtome sections were prepared in the usual way. More recently some excellent series of sections have been obtained by means of the new ester wax technique (Steedman, 1945). For detailed study, Heidenhain's haematoxylin and eosin was found to be the best combination of stains. Mallory's triple, and Masson's trichrome, stains both gave excellent results, and various specific stains were also used.

In attempting to determine the functions of the glands, two methods were adopted: comparison of sections, and experimental work. Sections of male, female, and young *Porcellio scaber*, and of specimens fixed at different stages of the moulting cycle, were examined in order to determine the correlation, if any, between gland development and reproduction, age and moulting respectively. Correlation with terrestrial life was investigated by comparing sections of a number of Isopoda with different habits. The following species were used:

*Limnoria lignorum* Rathke (marine).

*Idotea granulosa* Rathke (found at low-water mark).

*Ligia oceanica* Lin. (found at high-water mark).

*Asellus aquaticus* Lin. (fresh-water).

*Oniscus murarius* Cuv. (terrestrial, without tracheal organs).

*Porcellio scaber* Latr. (terrestrial, with tracheal organs).

*Armadillidium vulgare* Latr. (adapted to withstand still drier conditions).

*Hemilepistus klugii* Brdt. (adapted to withstand the extreme conditions of the sandy desert. Specimens of this species were kindly supplied by Professor H. G. Jackson).

Experimental work was carried out mainly on *Porcellio scaber*. Land Isopoda will live for long periods if kept in a damp atmosphere. Food is less important, and although they eat a great variety of substances, they are capable of living for several weeks without food. A diet consisting exclusively of fresh carrot, first suggested by Gunn (1937), and used more recently by Heeley (1941), is the most convenient for experimental work, and appears to provide the animals with all the

constituents necessary for normal health and activity. Numbers of *Porcellio*, *Oniscus*, and *Armadillidium* were kept in large museum jars containing soil, bark, moss, &c., and covered with a glass plate. A humid atmosphere was maintained by attaching a piece of moist blotting-paper to the under side of the glass plate. Single specimens were kept in Petri dishes, or in round tobacco tins, each containing a piece of moss, and having moist blotting-paper attached to the lid.

Later a special vivarium was made. It measures  $16'' \times 16'' \times 1\frac{1}{2}''$  and is divided into sections  $3''$  square by strips of wood  $1\frac{1}{2}'' \times \frac{3}{8}''$ . Each section is covered with a glass plate used in making lantern slides, and the base of the whole is covered with perforated zinc. Each section is numbered and contains a  $\frac{1}{4}''$  layer of bulb fibre. The vivarium is kept in a shallow zinc tray to which water can be added from time to time, and the fibre is kept damp by the absorption of water from the tray through the perforated zinc.

### III. HISTORICAL REVIEW.

Many workers have described the 'salivary glands' in various groups of Crustacea as ordinary racemose glands, with central ducts that unite to form a common trunk. Huet (1882) extended this theory to the point of suggesting that the small connective tissue cells attached to the surface of the glands are replacement cells, as in vertebrate salivary glands. He believed the glands to be present without exception throughout the Isopoda.

The first detailed account of these glands in the Isopoda was given by Ide (1891). He showed that their structure is quite different from that of the racemose type, each gland consisting of a regular mass of cells opening by chitinous canals into a central duct. They are always found, he states, in the neighbourhood of the mouth, either round the oesophagus or inside the mandibles. Ide made a special study of the glands in *Asellus*, two of which are larger than those of any other genus, but he also examined *Anilocra*, *Idotea*, *Oniscus*, and the parasitic *Gyge* and *Ione*. He observed considerable variation in the size and number of glands in related species (*Asellus*, for example, has only four rosette glands), and in

different parts of the same individual, and classified the genera accordingly.

In his monograph on *Ligia*, Hewitt (1907) describes two pairs of 'salivary glands' on each side of, and opening into, the oesophagus, each being made up of a large number of rosette glands. Ter-Poghossian's description (1909) agrees in general with that of Ide, except where he points out that in the terrestrial forms the rosette glands have a less restricted distribution than either Huet or Ide supposed. They are found, he states, throughout the whole of the head and its appendages. He found histological study very difficult in the land Isopoda, and was unsuccessful in his attempts to determine the course of the ducts.

The most detailed study of rosette glands has been made on those of Decapods. Farkas (1927), using *Astacus fluvialis*, divided the activities of the glands into four periods (of construction, rest, reconstruction, and destruction), and believed that, unlike most glandular structures, they never return to a former condition, but eventually degenerate.

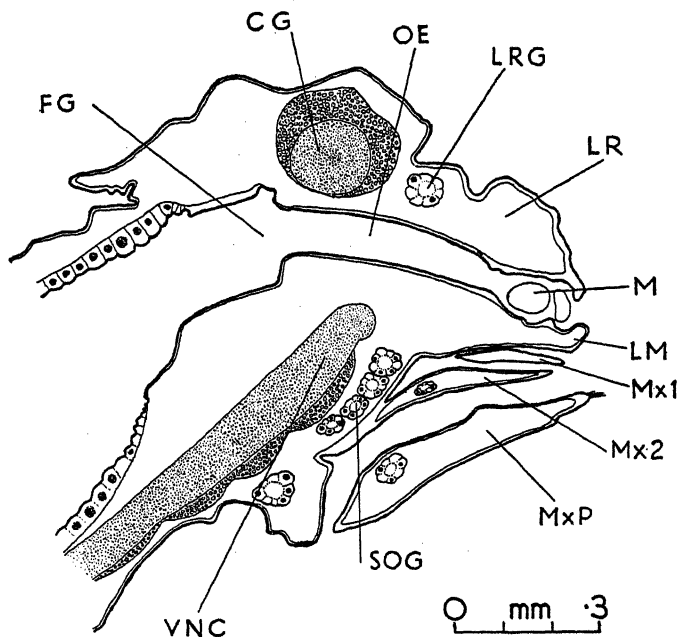
#### IV. DISTRIBUTION AND STRUCTURE.

A detailed study was made of the rosette glands in *Porcellio scaber* Latr., and the glands of other genera were then compared with these. Their ducts are extremely fine, and Ter-Poghossian was able to trace their course only in the case of the large glands of *Asellus*.

##### 1. *Porcellio scaber* Latr.

As mentioned above, the rosette glands in the land Isopoda are found only in the head and mouth-parts. In *Porcellio* a number of separate groups of glands may be distinguished. One group lies in front of the oesophagus at the base of the labrum, and comprises a single pair of glands (Text-fig. 1, LRG). These occupy the same position as the labral glands of *Asellus*, described by Ter-Poghossian, and are rather conspicuous in transverse section. Their ducts appear to run forwards towards the edge of the labrum. The majority of rosette glands are to be

found in two large groups, one at each side of the head (Text-figs. 2 and 3, LMG). Each group is somewhat flattened in a transverse plane. It extends from between the eye and second

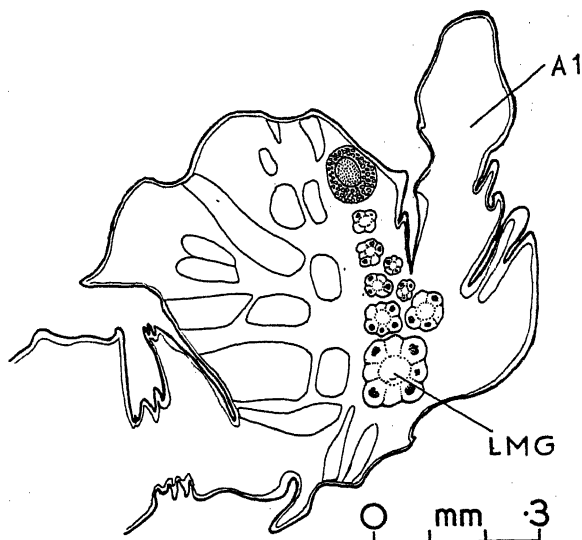


TEXT-FIG. 1.

Longitudinal section near middle line through head of young individual, showing rosette glands (the glands are more numerous in fully developed individuals). This and all the following figures refer to *Porcellio scaber* Latr. CG, cerebral ganglion; FG, foregut; LM, labium; LR, labrum; LRG, labral rosette gland; M, mandible; Mx1, first maxilla; Mx2, second maxilla; MxP, maxilliped; OE, oesophagus; SOG, suboesophageal rosette glands; VNC, ventral nerve cord.

antenna to the suboesophageal ganglia, and runs alongside, and lateral to, the peri-oesophageal commissure. The more ventral glands are usually much larger than the others (Text-fig. 2). The ducts from the glands in each group run together in a common trunk, but apparently do not actually unite (Text-fig. 3, DT). They open at the side of the labium (LM), where the

chitin is very thin. It was impossible to determine whether the ducts from all the glands in this region join the common trunk; some appear to open separately at the bases of the mouth parts. A fourth group is composed of a paired series of glands below



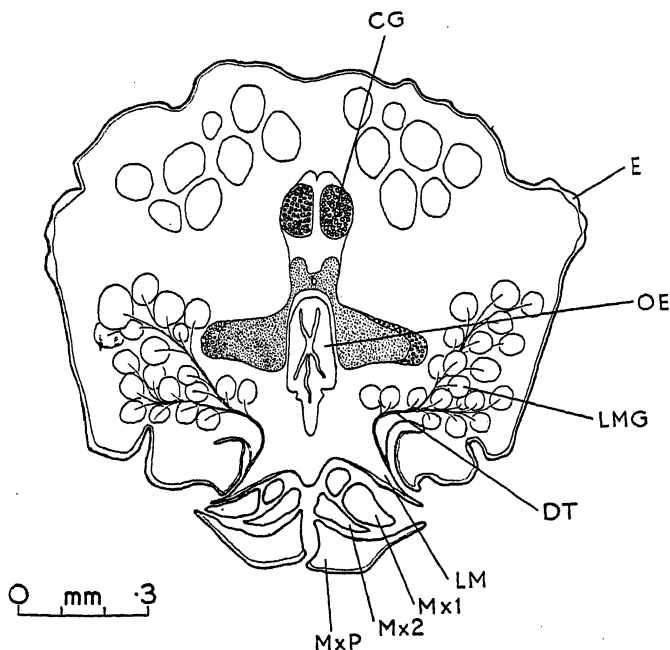
TEXT-FIG. 2.

Longitudinal section through lateral part of head from same individual as Text-fig. 1. A1, first antenna; LMG, rosette glands opening at side of labium.

the suboesophageal ganglia, above the bases of the mouth-parts, and extending into the labium (Text-fig. 1, *so*). Their ducts do not join those of the main group. Finally, rosette glands are found in the maxillipeds and second maxillae, where they occupy the proximal two-thirds of the appendage (Text-fig. 1). They are particularly abundant in the maxillipeds, and their ducts appear to run forwards towards the distal end of each mouth-part.

The rosette glands of *Porcellio* are very similar in structure to those of the Decapoda. Each consists of a spherical mass of cells surrounded by a delicate capsule of connective tissue (Text-fig. 6, *cr*). Nuclei of connective tissue cells can

sometimes be seen lying between the gland cells (Text-fig. 6, NCT). The cells composing each gland are relatively larger and fewer than in the Decapoda. Their nuclei, which lie in the



TEXT-FIG. 3.

Composite diagram of transverse section through head, showing arrangement of labial glands and their ducts. CG, cerebral ganglion; DT, trunk formed by ducts of labial rosette glands; E, compound eye; LM, labium; LMG, labial rosette glands; Mx1, first maxilla; Mx2, second maxilla; MxP, maxilliped; OE, oesophagus.

outer parts of the cells, are very large indeed (Text-fig. 6, NG), and each contains a conspicuous nucleolus. The central fibrillar substance seen in the glands of Decapoda is, in *Porcellio*, almost entirely obliterated by the gland cells, which extend almost to the centre of the gland. The latter is occupied by the end of the main duct (Text-fig. 8, MD). This is ampulla-shaped and relatively thick-walled. A number of thin-walled collecting

ducts (Text-fig. 8, CD) radiate from it to the gland cells, and in sections their openings can frequently be seen as small circles on the inner surface of the wall of the main duct. The statements of Ide and Ter-Poghossian concerning the number of collecting ducts are inaccurate. Single sections, such as the one figured in Text-fig. 8, suggest that each gland cell is provided with a separate collecting duct, and this is confirmed by examination of serial sections.

Although a large number of sections, prepared by a variety of fixing and staining methods, was examined, it was impossible to determine with certainty how the collecting duct communicates with the cytoplasm of the gland cell. The region of the cell immediately surrounding the collecting duct has a reticulate appearance, and stains more deeply with cytoplasmic stains such as eosin (Text-fig. 8, CA). It seems highly probable that, in the mature condition at least, a cluster of extremely fine, branching canals is formed in this part of the cell, and that these canals open in the collecting duct. A duct cell nucleus can always be seen (Text-fig. 6, ND). It lies nearer the centre of the gland than in the Decapoda, and is oval in shape, although somewhat crescentic in transverse section owing to its apposition to the duct. Other, more elongated, nuclei are found at intervals along the duct, their number apparently varying with its length.

## 2. Other Genera.

Rosette glands were found in all the Isopoda examined. They are similar to those of *Porcellio* in distribution and structure in *Oniscus*, *Armadillidium*, *Hemilepistus*, *Ligia*, and *Idotea*. In *Hemilepistus* and *Ligia* the cells of each gland are larger and fewer than those of *Porcellio*, while in *Idotea* they are smaller and more numerous. Ter-Poghossian's description of the glands in *Asellus* was confirmed. There are two pairs of very large rosette glands, one pair situated above the oesophagus and corresponding to the labral glands of *Porcellio*, and the other pair occupying the proximal half of the first maxillae. In *Limnoria* there are two large, many-celled rosette glands ventral to the foregut,



and from ten to twelve smaller ones at the base of the mouth-parts.

## V. FUNCTION.

### 1. Secretion of Digestive Fluid.

Various functions have been assigned to the rosette glands of the Decapoda, but in the Isopoda, owing to their restriction to the head region, they have always been assumed to be salivary glands. It was Ide's opinion, for instance, that their ducts pass to the cuticle of the mouth-parts, and that their secretion plays a part in digestion. He adds in support of this that they are better developed in parasitic forms, but this is a fallacious argument, since one of the most characteristic features of parasitic animals is the possession of a digestive system that is not better, but less well developed than that of their free-living allies.

Hewitt does not question the salivary function, while Ter-Poghossian points out that the glands occur most abundantly in the mouth region and mouth-parts, and appear to function as salivary glands since their secretion passes down the ducts and mixes with the food. There is no evidence in support of this assumption. Indeed, no salivary secretion appears to be necessary in the Isopoda (or in any Crustacean). It was shown by Murlin (1902) that the secretion of the 'hepatopancreas' contains enzymes which act on proteins, carbohydrates, and fats; while according to Nicholls (1931) the foregut of *Ligia* is merely an elaborate filter mechanism, digestion taking place entirely in the hepatopancreas. This has been found to be true also of *Porcellio*. It is very improbable, therefore, that the rosette glands function as salivary glands.

### 2. Formation of Cuticle.

The integument of the Decapod Crustacea consists of two layers, a thin superficial cuticle, and a much thicker underlying chitin. Yonge (1924, 1932) has shown that these two substances are quite distinct, and that while the chitin is formed by the chitinogenous epithelium, the cuticle is secreted by the tegumental glands. The evidence for the glands having this function

is threefold. In the first place the cuticle does not appear until after the formation of the new layer of chitin has begun, and then increases in thickness with it. Secondly the contents of the ducts of the tegumental glands have the same properties as the cuticle. Lastly there is a close correlation between the activity of the glands and the moulting cycle, the glands showing the greatest signs of activity shortly before ecdysis, when the cuticle is being formed most rapidly. After ecdysis the active glands degenerate and new glands appear. The secretion spreads out as a thin, continuous layer over the surface of the chitin, probably as a result of its low surface tension. The layer increases in thickness, and exposure after ecdysis apparently causes it to solidify.

The close similarity in structure between the rosette glands in Isopoda and the corresponding glands in Decapoda suggests that they may have the same function in both groups, and that the rosette glands of Isopoda may also be concerned with the formation of cuticle. Cuticle is undoubtedly present in the Isopoda, and forms a much thicker layer over the mouth-parts than over any other part of the body. Elsewhere it is very thin and in sections tends to break up and separate from the underlying chitin. The cuticle is thus best developed in precisely that part of the body in which the rosette glands are situated, and the possibility of these glands being concerned with cuticle formation was investigated by fixing specimens of *Porcellio scaber* Latr. at different stages of the moulting cycle, and sectioning in order to compare the conditions of the glands.

The moulting process in *Porcellio* occurs at irregular intervals, except in the case of young individuals and of breeding females (Heeley, 1941), and the cycle may be affected by external conditions as suggested in the case of *Asellus* by Unwin (1920). Ecdysis in the Isopoda takes place in two stages. The integument of the posterior half of the body (behind and including the 5th thoracic segment) is moulted first, that of the anterior half being shed two or three days later. By making longitudinal sections of an animal at the stage between posterior and anterior moults, it is possible to examine the conditions of the integument before and after moulting in the same section.

In the case of *Homarus* moulting occurs only once or twice a year, but the approach of ecdysis is indicated by the presence of gastroliths, which increase in size as the time of ecdysis draws near. The precise stage in the moulting cycle of an animal preparing to moult can therefore be readily determined. Gastroliths do not occur in the Isopoda, but an even more readily observable sign of approaching ecdysis is the appearance of white patches on the ventral side of the thorax. Herold (1913), who noted them in *Armadillidium*, believed them to be stores of lime, and thought that this formation of 'weissen Platten' was a means of getting rid of an excess of that substance. He also believed, however, that part of the lime is reabsorbed shortly before the moulting of the posterior half and used in hardening the new integument. This view was confirmed by Numanoi (1934), who worked on *Ligia exotica* and observed that after the posterior moult, the white plates on the thorax gradually fade away. Meanwhile, a chalky deposit appears on the pleopods, and after the anterior moult, this also fades. He concluded that the calcium for the new posterior integument is obtained from a temporary reservoir in the thoracic segments, and that the calcium for the new anterior integument is stored in a similar way in a temporary reservoir in the abdominal pleopods.

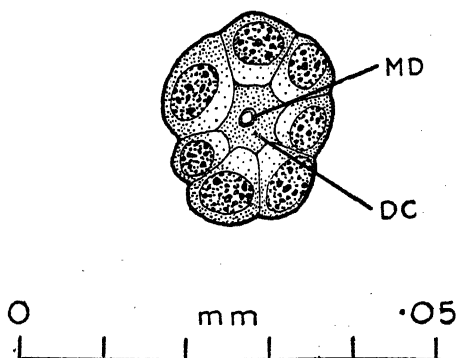
Thus it was a relatively simple matter to obtain sections of *Porcellio* that were fixed at various stages before and after ecdysis, and during the inter-moulting period. Young animals (approximately two thirds adult size) were used, since these, unlike mature individuals, moult at comparatively regular intervals of three to four weeks. Sections were prepared of specimens in which the white plates were entirely absent, slightly developed, half developed, and fully developed. Other individuals were isolated, allowed to moult, and then fixed at various periods after the moult (immediately after posterior moult, a few hours after posterior moult, immediately after anterior moult, a few hours after anterior moult, five, ten, and fifteen days after posterior moult).

The course of development and degeneration of the glands was traced by comparing the different appearances of the glands,

and arranging them in a progressive series (Text-figs. 4-11). The activity of the glands was then correlated with the stages of the moulting cycle. Furthermore, a comparison was made between the staining reactions of the gland contents and the cuticle.

(a) Development of the Rosette Glands.

In the earliest stage examined (Text-fig. 4), the nuclei occupy the outer part of the gland cells and are very large in proportion



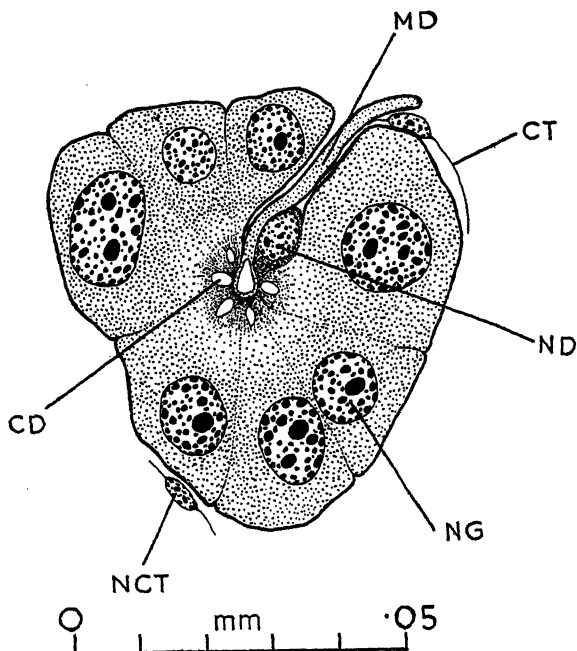
TEXT-FIG. 4.

Section through centre of young rosette gland from animal fixed a few hours after posterior moult. DC, duct cell; MD, main duct.

to the small size of the gland. The gland cells do not extend into the centre of the gland, as in the later stages, but surround what appears to be a very large duct cell (Text-fig. 4, DC). The main duct is clearly visible (MD), but no collecting ducts have apparently yet been formed. The cytoplasm of the gland cells has a granular appearance, and stains readily with eosin. The duct cell is less granular and less deeply stained. The gland increases in size, and the duct cell becomes both relatively and actually smaller (Text-fig. 5). The nuclei move towards the middle of the gland cells and collecting ducts appear. The cells later (Text-fig. 6) become differentiated into two regions: an outer, granular region, staining deeply with eosin as before, and an inner, paler region. The cytoplasm surrounding the collecting duct has now acquired a reticular appearance, staining

red with eosin. It is believed to consist of a system of minute, branching canals as already mentioned (Text-figs. 6 and 8, cA).

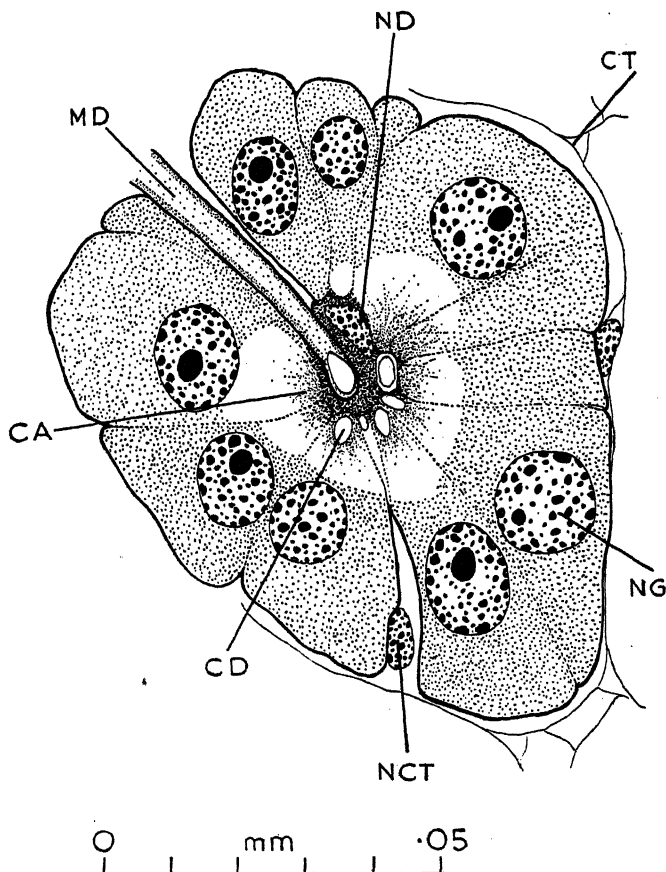
The gland has now reached its maximum size, and the nuclei, which are exceptionally large, even for gland cells, begin to



TEXT-FIG. 5.

Section through centre of half-developed rosette gland from animal fixed immediately after posterior moult. CD, collecting duct; CT, connective tissue; MD, main duct; NCT, nucleus of connective tissue cell; ND, nucleus of duct cell; NG, nucleus of gland cell.

show signs of activity. Small globules which stain a bright pink colour with eosin appear between the chromatin granules in the nucleus (Text-fig. 7, GL). This nuclear substance may also have the form of a single large globule, or may be granular, these differences in appearance probably representing successive stages in the formation of the secretion. The latter now passes out of the nucleus and forms a pink, granular mass in the cytoplasm at the outer end of the cell (Text-figs. 7 and 8,

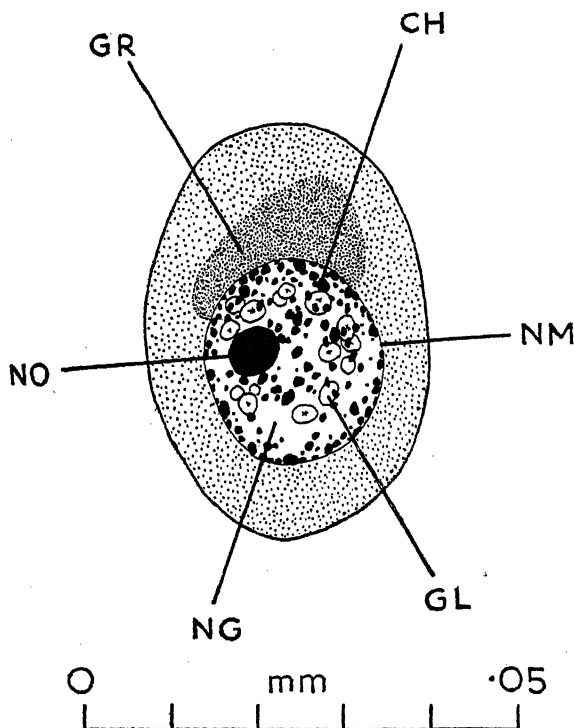


TEXT-FIG. 6.

Section through centre of immature rosette gland from animal fixed immediately after posterior moult. CA, fine canals in cytoplasm; CD, collecting duct; CT, connective tissue; MD, main duct; NCT, nucleus of connective tissue cell; ND, nucleus of duct cell; NG, nucleus of gland cell.

GR). This gradually disappears, and the cytoplasm becomes vacuolated and stains increasingly deeply with haematoxylin (Text-fig. 8, s). In the mature gland (Text-fig. 9) the cytoplasm is full of secretion and stains very darkly.

After this the gland begins to degenerate, the cytoplasm no longer has any affinity for either eosin or haematoxylin and the cells appear to be pouring their secretion into the duct.

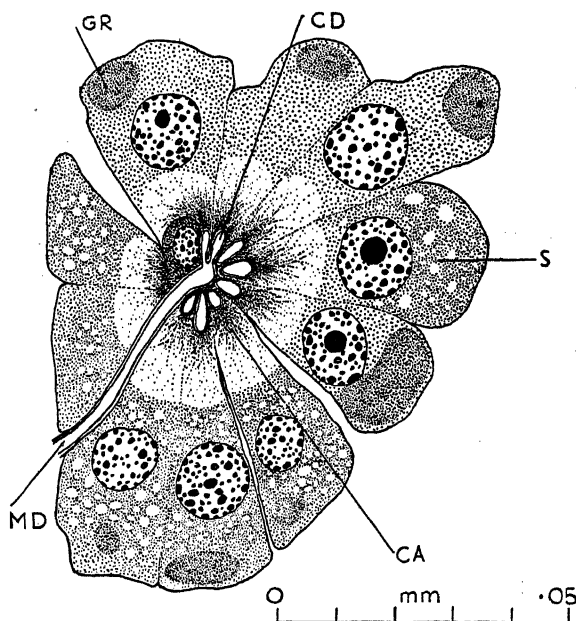


TEXT-FIG. 7.

Section through single cell of rosette gland from animal fixed immediately after posterior moult, showing formation of secretion inside nucleus. CH, chromatin granules; GL, globule of secretion inside nucleus (stained bright pink with eosin); GR, finely granular mass of secretion in cytoplasm (stained red with eosin); NG, nucleus of gland cell; NM, nuclear membrane; NO, nucleolus.

This emptying process begins at the inner end of the cell (Text-fig. 10, cr), and extends outwards. Finally (Text-fig. 11), the whole of the cytoplasm takes on a gray and coarsely granular appearance, collecting ducts are no longer visible, and the nuclei, obviously degenerating, now have a shrunken appear-

ance and pass to the outer edge of the cell. The gland then presumably breaks up and disappears.



TEXT-FIG. 8.

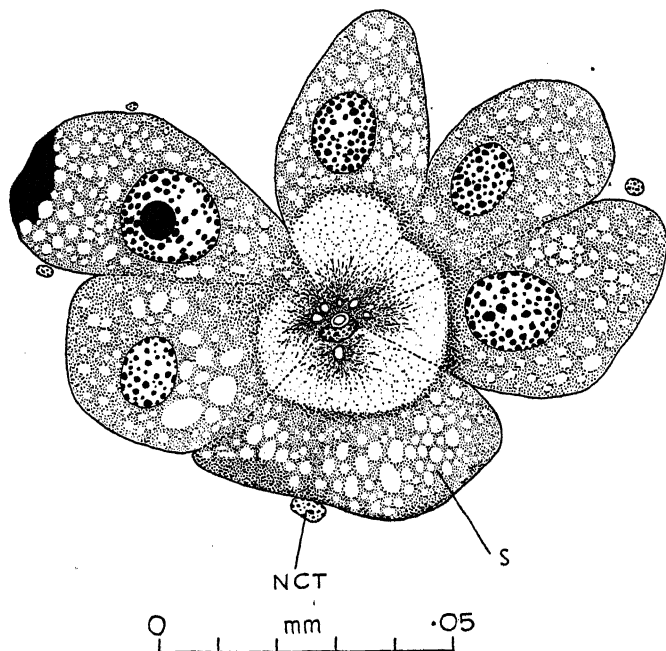
Section through centre of rosette gland during formation of secretion from animal fixed immediately after posterior moult. CA, fine canals in cytoplasm; CD, collecting duct; GR, finely granular mass of secretion forming in cytoplasm (stained red with eosin); MD, main duct; s, darkly staining secretion forming in cytoplasm (stained dark blue or black with haematoxylin).

#### (b) Correlation with the Moulting Cycle.

Mature glands, filled with secretion, were observed only in material fixed ten days after the posterior moult, or in individuals with half developed white plates. In other words, the glands are fully developed and ready to pour out their secretion at a stage approximately half-way through the moulting cycle, which in the case of the immature animals used lasts three to four weeks. At fifteen days after moulting, most glands had the half-empty appearance of the one shown in Text-fig. 10.



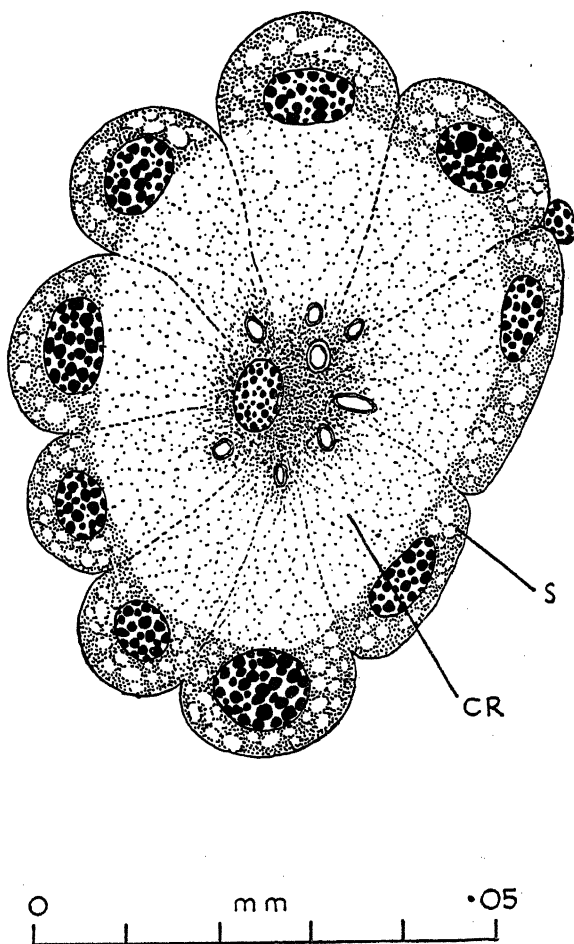
Completely empty glands are present immediately after the posterior moult, and degenerating glands a few hours later. Young glands, similar to that shown in Text-fig. 4, were seen only



TEXT-FIG. 9.

Section through centre of mature rosette gland in state of active secretion from animal fixed ten days after posterior moult. NCT, nucleus of connective tissue cell; s, darkly staining secretion forming in cytoplasm.

immediately after posterior moult, and in the stage fixed a few hours later. Immature glands were also seen at these stages, and at all subsequent stages until ten days after moulting, when all the glands had reached maturity. It may be noted here that formation of the new integument precedes by several days the actual process of ecdysis, and that it is in sections of this latter stage, approximately half-way through the moulting cycle, that the new integument first begins to appear.



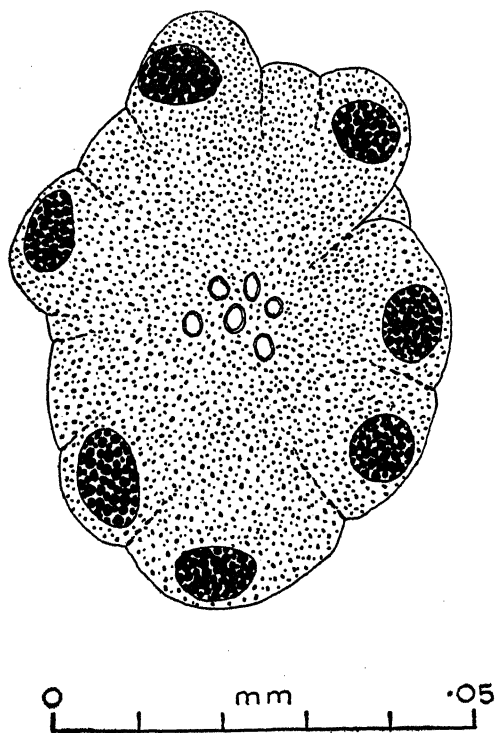
TEXT-FIG. 10.

Section through centre of rosette gland which has partly discharged its secretion from an animal fixed fifteen days after posterior moult. CR, clear region of cell from which secretion appears to have been discharged; S, darkly staining secretion in cytoplasm.

(c) Staining Reactions.

Both the cuticle and the secretion in the glands are stained black with iron haematoxylin, while chitin is unstained. With

Mallory's triple stain, the cuticle, the duct contents, and the innermost region of the gland cells stain bright red, chitin blue. Masson's trichrome stain colours the cuticle and glands bright



TEXT-FIG. 11.

Section through centre of rosette gland which is degenerating after discharging its secretion from animal fixed fifteen days after posterior moult.

red as with Mallory, and the chitin green. Thionin and mucicarmine gave negative results, but with Best's carmine the glands are stained red. This indicates the presence of glycogen, but whether this is an essential constituent in the formation of cuticle, or merely a source of energy needed in the elaboration of this, is not certain.

Pryor (1940) has shown that the epicuticle of insects is formed by the interaction of two substances: a water-soluble protein, and a dihydroxyphenol. It is possible that crustacean cuticle may be formed in a similar way. The presence of a polyphenol may be demonstrated by the argentaffin test (Lison, 1936). This test was applied to sections of individuals fixed during the period when the glands are actively secreting, but neither cuticle nor glands gave positive results.

#### (d) Discussion.

It is evident from an examination of the rosette glands fixed at different stages of the moulting cycle, that the glands are actively secreting for only a few days before moulting is due to take place. (Their early maturity, half-way through the moulting cycle, is not surprising when it is remembered how short this cycle is in comparison with that of the Decapoda.) At no stage other than between posterior and anterior moults are degenerating and newly formed glands seen. After ecdysis the old glands degenerate and are destroyed. At the same time new ones appear, and these subsequently develop and show greatest signs of active secretion at the time when cuticle is most rapidly being formed. There is therefore a very definite correlation between the activity of the rosette glands in *Porcellio* and the moulting cycle, and it is clear that they are intimately connected with ecdysis.

Since the glands are in a state of active secretion only during that period in the moulting cycle when new cuticle is being laid down, and since the rosette glands in *Porcellio* bear such a close structural resemblance to those of the Decapoda, it appears highly probable that their function is the secretion of the cuticle. This view is supported by a comparison of the staining reactions of the cuticle and of the contents of the mature glands and their ducts. The affinity for stains is invariably the same, and in no instance does the glandular secretion show a reaction that is not also shown by the cuticle. Furthermore, as in the Decapoda, the cuticle first appears after the formation of the new chitin has begun, and then increases in thickness with it. This process may most easily be explained

on the assumption that, while the chitin is formed entirely by the chitinogenous epithelium, the cuticle is secreted by the rosette glands as a fluid substance, which is carried through the newly formed layer of chitin by the ducts. Finally, the rosette glands in *Porcellio* are confined to the head region, and here (and particularly in the mouth-parts, on or near which the ducts open) the cuticle is considerably thicker than in any other part of the body.

A similar inequality in the distribution of the cuticle is found in the Decapoda, for although the rosette glands are present under the integument in all parts of the body in this group, they are more abundant in the head region than elsewhere, and the cuticle is especially thick round the mouth, on the labrum, and in the oesophagus. This unequal distribution of the cuticle is apparently correlated with the function performed. Crustacean cuticle appears to have three main functions (Yonge, 1936): (1) protection of the new chitin from chemical action during dissolution of the old chitin in the early stages of ecdysis (Yonge suggests that this may have been its primary function); (2) protection of the chitin (which is a relatively soft and delicate substance) from mechanical and abrasive action; (3) control of permeability (unimpregnated chitin is freely permeable). The cuticle has in addition acquired certain subsidiary functions in the Decapoda, including the formation of the attachment membranes of the eggs (Yonge, 1935, 1937) and of the cement which secures the statoliths to the sensory setae (Lang and Yonge, 1935). It has also been used for a variety of purposes in other groups.

The first of these functions, namely the protection of the new chitin from chemical disintegration during ecdysis, will be performed as effectively by a thin layer of cuticle as by a thicker layer. The relatively thin cuticle covering the greater part of the body in *Porcellio*, which is presumably formed by the fluid secretion from the rosette glands in the head region spreading backwards as a result of its low surface tension, would thus appear to be as efficient in this respect as the thicker cuticle of the Decapoda, in which the rosette glands are universally distributed below the integument.

In both groups mechanical protection of the integument is provided partly by the cuticle and partly by calcification of the underlying chitin. Mechanical protection is especially important in the region of the mouth-parts and foregut. These have to withstand considerable abrasive action in dealing with a diet which, in the Decapoda, frequently includes hard, sharp, heavily calcified materials, and which, in the Isopoda, is largely made up of the tough, dry food substances associated with life on dry land. It is essential, however, that the means of protection should allow for free movement of the mouth-parts, and stretching of the wall of the oesophagus during the passage of food. This requirement is not fulfilled by calcification, which has the effect of increasing to a considerable extent the hardness and rigidity of the material involved. In both groups, therefore, the integument in the region of the mouth is protected by a greatly thickened cuticle, which is both flexible and elastic, while elsewhere the cuticle is thinner, and protection is mainly dependent on a high degree of calcification.

Control of permeability is a more serious problem for land animals, particularly in its effect on water loss, and the reduced condition of the cuticle over the greater part of the body in *Porcellio* is a little more difficult to understand. Impregnation of the integument with calcium salts, however, is of considerable importance in this respect, and probably goes a long way towards preventing desiccation. Moreover, there is evidence which suggests that the lobed glands produce a secretion which reduces water loss still further, and which may be largely responsible for the success of the Isopoda in their colonization of dry land. This evidence will be discussed in a later paper.

It is evident, therefore, that the rosette glands in *Porcellio* are concerned with the formation of cuticle, and the rosette glands of other Isopoda, since they have, in general, a similar distribution and structure, may readily be assumed to have the same function.

## VI. SUMMARY.

1. The land Isopoda possess five (or possibly six) distinct varieties of tegumental gland, and these are briefly described.

Of these, only the rosette glands are known to occur outside the group.

2. The rosette glands of the Isopoda are similar to those of the Decapoda, but are confined to the head and mouth-parts. The structure and development of the rosette glands in *Porcellio scaber* Latr. are described.

3. Activity is intimately connected with the moulting cycle, the glands reaching maturity and liberating their secretion only during the few days before ecdysis.

4. The contents of the glands and their ducts show the same staining reactions as the cuticle.

5. The cuticle first appears after the formation of new chitin has begun and increases in thickness with it.

6. The thickness of the cuticle is greatest in the mouth-parts, where the ducts of the rosette glands open.

7. The function of the rosette glands in the Isopoda is similar to that of the corresponding glands in the Decapoda, namely to secrete the layer of cuticle on the surface of the integument.

8. Seven other species of Isopoda, from a wide range of habitat, were examined. Rosette glands similar in structure and distribution to those of *Porcellio*, and presumably having the same function, were found in every case.

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# The Chondrocranium of *Calotes versicolor* (Daud.) with a Description of the Osteocranium of a just-hatched Young

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With 16 Text-figures

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(c) Embryo, head-length 7.0 mm.		
(d) Embryo, head-length 8.0 mm.		
II. Osteocranium of a just-hatched young of <i>Calotes versicolor</i> (Daud.), head-length 8.0 mm.		

## INTRODUCTION

THE chondrocranium of a number of lizards has been described, viz. *Lacerta* (Leydig, 1872; Parker, 1880; Gaupp, 1900, 1906; de Beer, 1930); *Ascalabotes* (Sewertzoff, 1900); *Hemidactylus*, *Platydictylus* (Versluys, 1908); *Anguis* (Zimmermann, 1913); *Eumeces* (Rice, 1920); *Lygosoma* (Pearson, 1921); *Platydictylus* (Hafferl, 1921); *Lygodactylus*, *Pachydactylus*, and *Agama* (Brock, 1932); *Ablepharus* (Haas, 1935). In the descriptions of the adult skull of *Chalcides* (Haas, 1936) and *Adontias* (de Villiers, 1939) persistence of chondrocranial cartilages in various regions is also noticed. Boulenger (1890) gives figures of lateral, dorsal, and ventral aspects of the adult skull of *Calotes jubatus* (Dum and Bibr.) which are also reproduced in the latest edition of "Sauria" in "Fauna of British India" (Smith, 1936). The columella

auris has been studied in *Lacerta* (Hoffmann, 1889; Versluys, 1898, 1903; Cords, 1909; Dombrowsky, 1918, 1924), and the adult hyoid apparatus of *Calotes versicolor* is described by Gnanamuthu (1937) and Narayanaswamy Iyer (1943).

I propose to describe in detail the fully formed chondrocranium of *Calotes versicolor* (Daud.) while making references to important points in three earlier stages, and as well the osteocranium of a just-hatched form. The adult skull is described by Narayanaswamy Iyer (1943).

#### MATERIAL AND METHODS

The following are the stages of *Calotes versicolor* (Daud.) studied:

Head-length.	3.6 mm.	} Embryos.
„	6.0 mm.	
„	7.0 mm.	
„	8.0 mm.	
„	8.0 mm.	Just-hatched young.

The fully formed chondrocranium described belongs to an embryo of a head-length of 8.0 mm.; the head of the just-hatched lizard also measured the same. Alizarin transparencies of 8.0 mm. embryo and just-hatched young, van Wijhe's preparations of all the stages and wax models of the chondrocranium of the embryonic stages have been made.

I wish to express my sincere thanks to Professor E. S. Goodrich for having gone through the paper and for helpful criticism, and also to Professor A. Subba Rau for lending Gaupp's paper on *Lacerta* from his personal library.

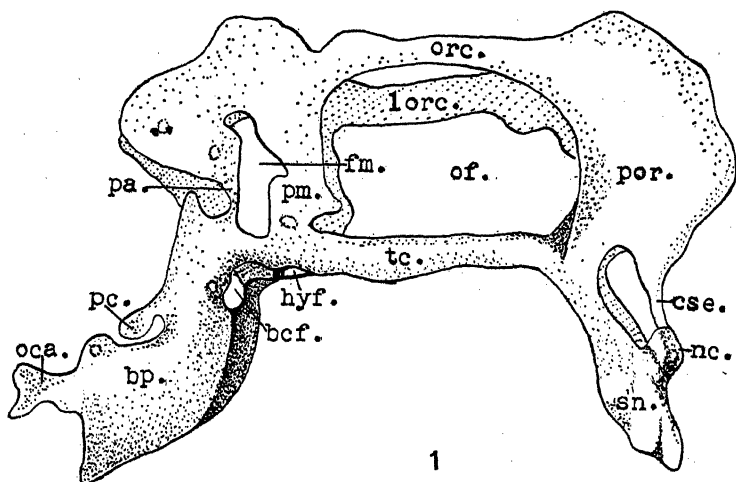
#### OBSERVATIONS

##### *I a. Embryo, H.-L. 3.6 mm. (Text-fig. 1).*

In the occipital region, the occipital arch (Text-fig. 1, *oca*) arising from the basal plate (*bp*) is unconnected by a tectum.

The otic capsule is not yet formed but for the slight formation of cartilage in the region of the future recessus scalae tympani lateralis (metotic fissure). The independent cochlear cartilage

(*pc*) is united with the basal plate by a basicochlear commissure; while on one side a prefacial commissure is noticed, on the other the facial root is unenclosed. The basal plate shows one hypoglossal foramen on one side and two on the other.



TEXT-FIG. 1.

The chondrocranium of a 3.6-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 50$  (model). *bcf*, basicranial fenestra; *bp*, basal plate; *cse*, commissura sphenoethmoidalis; *fm*, fenestra metoptica; *hyf*, hypophysial fenestra; *lorc*, left orbital cartilage; *nc*, cartilage of nasal capsule; *oca*, occipital arch; *of*, optic fenestra; *orc*, right orbital cartilage; *pa*, pila antotica; *pc*, prominentia cochlearis; *pm*, pila metoptica; *por*, preoptic root; *sn*, septum nasi; *tc*, trabecula communis.

There is an interesting feature in the condylar region. The notochord is dorsal to the basal plate. The occipital arch is united with the basal plate and represents the neural arch (*bd*) of the proatlas vertebra; the hypocentrum (*bv*) of this fuses with the basal plate to form the single condyle. Normally this must fuse with the pleurocentrum (*id*) behind it to form a vertebra. However, in lacertilians, in the condylar region this pleurocentrum fuses instead with the pleurocentrum of the atlas and both with the same of the axis vertebra which forms the odontoid process. That the occipital condyle is formed by

the hypocentrum of the proatlas, which at least is temporarily united with its own pleurocentrum, is seen in this stage of *Calotes* (Text-fig. 15 r). Here the fusion is seen between the basal plate (inclusive of the hypocentrum of the proatlas vertebra), the pleurocentrum of proatlas and atlas vertebrae and that of the axis. A similar phenomenon has been described in *Eumeces*, where Rice (1920) recorded the confluence of the cartilage of the odontoid process and the basal plate. The occipito-atlantal joint is, therefore, intravertebral in lacertilians.

In the orbitotemporal region the orbital cartilages (Text-fig. 1, *orc*) are paired and separate. Extending from the posterior region of the pila antotica (*pa*) with which each orbital cartilage is united, it ends anteriorly as a short projection in front of its union with the preoptic root (*por*) and shows a number of fenestrae due to absence of chondrification in the wall. In front of the pila antotica (*pa*) arising from the lateral aspect of the crista sellaris, the pila metoptica (*pm*) of each side unites with the orbital cartilage and between the two pilae there is the metoptic fenestra (*fm*) in which the oculomotor nerve gains exit either through an incomplete or complete orifice in the pila metoptica (*pm*). The trochlear nerve gains exit between the pila antotica and the orbital cartilage, i.e. in the dorsal part of pila metoptica or through an orifice in the orbital cartilage. The abducens nerve passes out anteriorly to the incisura prootica and becomes extracranial in the metoptic region.

The trabecula communis (*tc*) formed anteriorly to the hypophysial fenestra (*hyf*) is continuous with the unfenestrated nasal septum (*sn*); the orbital cartilage widens in the region of the nasal septum and unites with the trabecula by means of the preoptic root (*por*). Further anteriorly, the sphenethmoid commissures (*cse*) unite the orbital cartilages with the nasal capsule (*nc*).

A parietotectal cartilage is feebly represented.

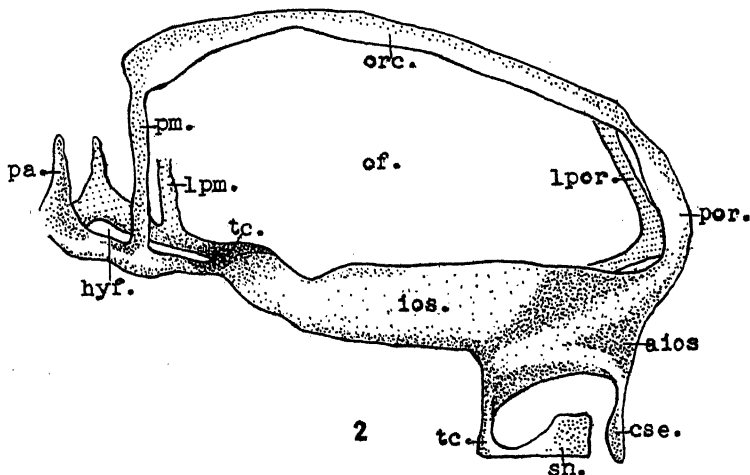
A sclerotic cartilage is formed.

The processus pterygoideus is still in a dense mesenchymatous condition and a processus ascendens is not formed, so also the basitrabecular process; a meniscus pterygoideus is recognizable as a deep mass of mesenchyme.

Meckel's cartilage is well developed, a feature also noticed in other lizards.

I b. Embryo, H.-L. 6.0 mm. (Text-fig. 2).

It was not possible to secure stages between the previous and the present.



TEXT-FIG. 2.

The chondrocranium of a 6.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 50$  (model); otic capsules and a part of the nasal capsule not included. *aios*, anterior portion of interorbital septum; *cse*, commissura sphenoethmoidalis; *hyf*, hypophysial fenestra; *ios*, interorbital septum; *lpm*, left pila metoptica; *lpor*, left preoptic root; *of*, optic fenestra; *orc*, right orbital cartilage; *pa*, pila antotica; *pm*, right pila metoptica; *por*, right preoptic root; *sn*, septum nasi; *tc*, trabecula communis.

A tectum of the occipito-auditory region is not formed.

The cochlear portion and the semicircular canal cartilages are united by the basicochlear commissure as in the previous stage; a basivestibular commissure is not formed. The fenestra ovalis and basicapsular fenestra are confluent. The foot plate of the columella is seen as an independent mass of cells. Between the posterior semicircular canal portion and the occipital arch, in the fissura metotica, the IX, X, and XI cranial nerves

gain exit, while in the basal plate the two hypoglossal foramina are seen. A prefacial commissure is also noticed.

In the orbitotemporal region, the oculomotor and trochlear nerves gain exit through the metoptic fenestra; the abducens cuts the base of the pila antotica and passes through a tunnel in the basal plate for a short distance. The crista sellaris unites the parachordals in the region posterior to the hypophysial fenestra.

An interorbital septum (Text-fig. 2, *ios*) has appeared which posteriorly is continuous with the trabecula communis (*tc*) in front of the hypophysial fenestra (*hyf*). Anteriorly the septum forks and from the dorsal ends of the fork start the preoptic roots (*por*, *lpor*) and each root is met by the orbital cartilage of its side. The olfactory ends of the forks are joined by the sphenethmoid commissures (*cse*). Thus between the preoptic roots and the sphenethmoid commissures, as it were, the anterior portion of the interorbital septum (*aios*) has been added in the dura mater. The pila metoptica (*pm*, *lpm*) is paired and united with the orbital cartilages (*orc*) as in the previous stage. There is a single large optic fenestra (*of*) between the interorbital septum and orbital cartilages. As in the previous stage, the orbital cartilage does not project posteriorly to the metoptic pillar.

The unchondrified processus pterygoideus is shorter than in the previous stage and the cartilaginous processus ascendens is well developed with a free meniscus pterygoideus on its medial side. A basitrabecular articulation is also developed.

In the nasal region the nasal septum is well formed and dorsally it is united with the parietotectal cartilage. The sphenethmoid commissure unites with the paranasal cartilage posteriorly.

The sphenethmoid commissure must unite with the antorbital cartilage, but since the latter is not clearly demarcated, it is stated that the commissure unites with the paranasal cartilage. Between the paranasal and the parietotectal cartilages in a large gap the lateral nasal branch of the ethmoid nerve gains exit; thus, the epiphaneal foramen is not yet enclosed.

The nasal septum (*sn*), which is not continuous with the interorbital septum (*ios*), shows ventrally a clubbed appearance;

this thickening continues posteriorly and ends where it meets the interorbital septum. In *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920) it is noticed that the trabecula communis continues anteriorly and unites with the base of the nasal septum and could be easily differentiated from the interorbital septum by its rounded appearance. In *Calotes*, unlike the previous stage, the trabecula communis cannot be differentiated as in *Lacerta* or *Eumeces*.

The hyobranchial skeleton is laid down but will be described later (8.0 mm. stage). There is no connexion between the hyoid cornu and the columella.

I c. Embryo, H.-L. 7.0 mm. (Text-fig. 3).

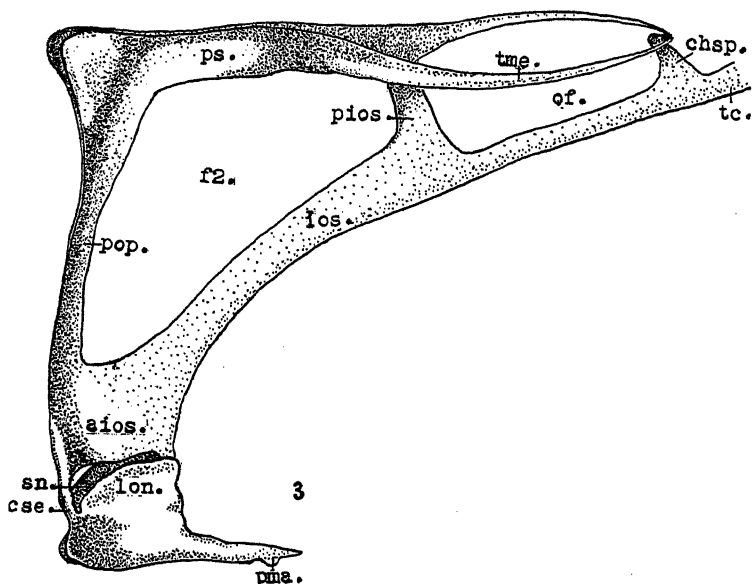
The cartilages of the occipital, otic, olfactory, the columella auris and the hyoid apparatus are well formed and will be described in the next stage.

A tectum synoticum plus posterior is noticed in this stage uniting the otic capsules and occipital arches. A short processus anterior tecti is given off from the tectum.

At the region the sphenethmoid commissure (Text-fig. 3, *cse*) unites with the interorbital septum (*ios*), the forked nature of the interorbital septum seen in the previous stage is lost, and the two commissures, therefore, arise from a thick cartilage; from the dorsal end of the septum, the two preoptic roots, which have united together to form a pillar, arise. I have called this the preoptic pillar (*pop*). From the upper end of this pillar is noticed the flooring cartilage for the brain, the planum supra-septale (*ps*) as in *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920). From the posteroventral aspect of the planum the two orbital cartilages, now called taenia medialis (*tme*), proceed posteriorly, and at the region where the taeniae start from the planum, there is an obliquely vertical pillar of cartilage (*pios*). This pillar, not noticed in previous stages, arises from the interorbital septum (*ios*) below, separating an anterior septal fenestra (*f2*) from a posterior optic fenestra (*of*). These paired taeniae mediales (*tme*) unite posteriorly; and slightly in front of this union, the united cartilago hypochiasmatica, subiculum infundibuli and pilae metopticae (*chsp*), arising from the trabecula communis



(*tc*) in front of the hypophysial fenestra, unite with the conjoint taeniae. Obviously the taeniae posterior to the united vertical cartilage referred to above represent the reminiscent orbital



TEXT-FIG. 3.

The interorbital septal region of a 7.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 50$  (model). *aios*, anterior portion of interorbital septum; *chsp*, pillar formed by the union of cartilago hypochiasmatica, subiculum infundibuli, and pilae metopticae; *cse*, commissura sphenoethmoidalis; *f2*, the larger septal fenestra; *ios*, interorbital septum; *lon*, lamina orbitonasalis; *of*, optic fenestra; *plos*, pillar from the interorbital septum to the posterior portion of planum supraseptale; *pma*, processus maxillaris anterior; *pop*, pillar formed by the fusion of the two preoptic roots; *ps*, planum supraseptale; *sn*, septum nasi; *tc*, trabecula communis; *tme*, taenia medialis.

cartilage which connected the pilae metopticae and antotica in the previous stage.

The incomplete fenestra behind the united vertical cartilage (*chsp*) and in front of the pila antotica represents the metoptic region.

*I d.* Embryo, H.-L. 8.0 mm. (Text-figs. 11-14).

The fully formed chondrocranium belongs to this stage.

### 1. The Basal Plate and Notochord.

The basicranial fenestra (Text-fig. 2, *bcf*) is noticed, as already described, from the 3.6-mm. stage and is not formed by the resorption of cartilage in this region. The basicapsular and basivestibular commissures are formed (also noted in the previous stage) and the foramen ovalis is limited by the growth of cartilage in the ovalis region of the otic capsule. In the 3.6-mm. stage, the basicapsular fenestra was confluent with the fenestra ovalis. I cannot say when exactly the otic capsule becomes united with the parachordals, not having the stages between 3.6 mm. and 6.0 mm.

From the basicranial fenestra, the basal plate (Text-fig. 11; Text-fig. 12, *bp*) gently rises up towards the foramen magnum (*fma*); anterior to the hypophysial fenestra (Text-fig. 12, *hyf*) the interorbital septum (Text-fig. 11, *ios*) forms a deep arch near the olfactory capsule before uniting with the nasal septum (*sn*). There is a broad prefacial commissure (*pfcc*). The pila antotica (Text-figs. 11, 12, *pa*) arises from the anterolateral corner of the basal plate, and in this region the two sides are connected, in front of the basicranial fenestra (Text-fig. 12, *bcf*), by the crista sellaris (*cs*). The abducens nerve pierces the basal plate internally to the pila antotica (*pa*), passes through a tunnel for a short distance, and then emerges in the basal plate by an orifice (*fa*). In the condylar region the notochord (*nc*) runs dorsally in the reniform condyle seen both in 7.0-mm. and 8.0-mm. stages. This condyle is situated between two projections (Text-figs. 11, 12, *oc*) of the basal plate and later the two projections and condyle fuse to give rise to a monocondyle. Even in the adult *Calotes*, the line of fusion of the hypocentrum between the two exoccipital portions is clearly visible. In *Eumeces* Rice (1920) described a deep notch between two projecting condyle-like cartilages which he compared with the mammalian bicondyle; in *Lacerta* (Gaupp, 1900) the two projections are not so prominent.

The notochord in *Calotes* is wedged in the basal plate except posteriorly, where it runs dorsally; where it enters the odontoid process, it passes through the basal plate for a very short distance. Anteriorly it extends into the basicranial fenestra as far as the crista sellaris (not shown in my figures), as in other lizards.

## 2. The Occipital Region.

The two principal occipital arches (Text-fig. 11; Text-fig. 12, *oca*) are united dorsally above the foramen magnum (*fma*) by the tectum synoticum (plus tectum posterius) (*tsy*) which also connects the otic capsules. While three preoccipital arches spring from the basal plate on each side in front of the occipital arch enclosing three hypoglossal foramina in *Lacerta* (Gaupp, 1900), there are only two pairs of hypoglossal foramina (Text-fig. 11, *hy*) in *Calotes*. Between the otic capsule and the occipital arch there is the jugular foramen (Text-figs. 13 A, 13 B, *fj*) for the exit of the X and XI cranial nerves and jugular vein (corresponding to the posterior portion of the fissura metotica of *Lacerta* (Gaupp, 1900)). This jugular foramen is practically separated from the more anterodorsal recessus scalae tympani lateralis (*rsl*) by the coming together of the ampullary portion (*ppsc*) of the posterior semicircular canal (*psc*) and the basal plate (*bp*); the connective tissue between these two is so thin that the fissura metotica may be studied in two parts, viz. an anterior recessus scalae tympani lateralis and a posterior jugular foramen. Such a separation is also seen in *Lacerta* (Gaupp, 1900), while in *Eumeces* (Rice, 1920) is described an anterior recessus scalae tympani and a posterior foramen jugularis, being formed by the apposition of the posterior ampullary prominence and the basal plate in front of the anterior hypoglossal foramen.

The jugular foramen itself is cut up into a larger anterior (Text-fig. 13 A, *fj*) and a tiny posterior portion (*fj'*) between the occipital arch and the otic capsule, the separation being due to the coming together of otic capsule and the occipital arch. This division is of no significance since no nerves leave the cranium through the posterior orifice. An individual variation of the

metotic fissure in *Lacerta* where it is completely cut into two parts is also noted by Gaupp (1900, Fig. 4).

### 3. The Auditory Region.

The otic capsules are large and are as long as the basal plate (Text-figs. 13A, 13B, *bp*); posteriorly there is the tectum synoticum united with the tectum posterius (Text-fig. 12, *tsy*). From the anterior margin of the tectum, there arises medially a cartilaginous process, the processus anterior tecti (*pat*) supporting the endolymphatic organ. The united basicapsular and basivestibular commissures connect the basal plate with the otic capsule, leaving a large gap for the facial nerve (Text-fig. 11; Text-figs. 13A, 13B, *ff*).

**External features.**—Externally each otic capsule exhibits the following features. The cochlear portion (Text-fig. 13A, *pc*) is longer than in *Lacerta* (Gaupp, 1900); and, if a line is drawn connecting the facial foramen (*ff*) to the anterior end of the recessus scalae tympani lateralis (*rsl*), a large portion of the cochlear prominence is cut off as in *Eumeces* (Rice, 1920). The topography of the remaining parts agrees fairly with that in *Lacerta* and *Eumeces*. Laterally the three semicircular canals, viz. the most clearly marked out anterior semicircular canal (*asc*) which is completely separated for a short distance by a gap (*gapc*) from the other two, the prominent posterior semicircular canal (*psc*) separated near the sinus superior (*ss*) by the gap referred to above, and the lateral semicircular canal (*lsc*) forming also a ridge-like prominence, are noticed. Connected with the anterior or inferior openings of these semicircular canals, the ampullary prominences (*pasc*, *plsc*, *ppsc*) are also discoverable; the ampullary prominence at the inferior opening of the posterior semicircular canal is not very prominent.

From the prominence of the lateral semicircular canal (*lsc*) is seen a projection, the crista parotica (*cp*), with which another cartilaginous structure, the processus paroticus (*pp*), is continuous. Anteroventrally to these projections, there is the large fenestra vestibuli or ovalis (*fo*) in the cochlear wall. An unchondrified portion (*uc*) in the wall near the processus paroticus is also noticed.

On the medial aspect of the otic capsule (Text-fig. 13B) a large foramen acusticus anterior (*faa*) and a larger foramen acusticus posterior (*fap*) are noticed. Near the anterior foramen in the basal plate (*bp*) is the facial foramen (*ff*) referred to above. Anterior to this is the prominentia recessus utriculus (*pru*), the wall of which unites with the basal plate forming the prefacial commissure (*pfc*). The endolymphatic foramen (*ef*) is small, unlike that in *Eumeces* (Rice, 1920), and is posterior to the foramen acusticus posterior. Some unchondrified portions (*uc*) in the form of foramina are also noticed.

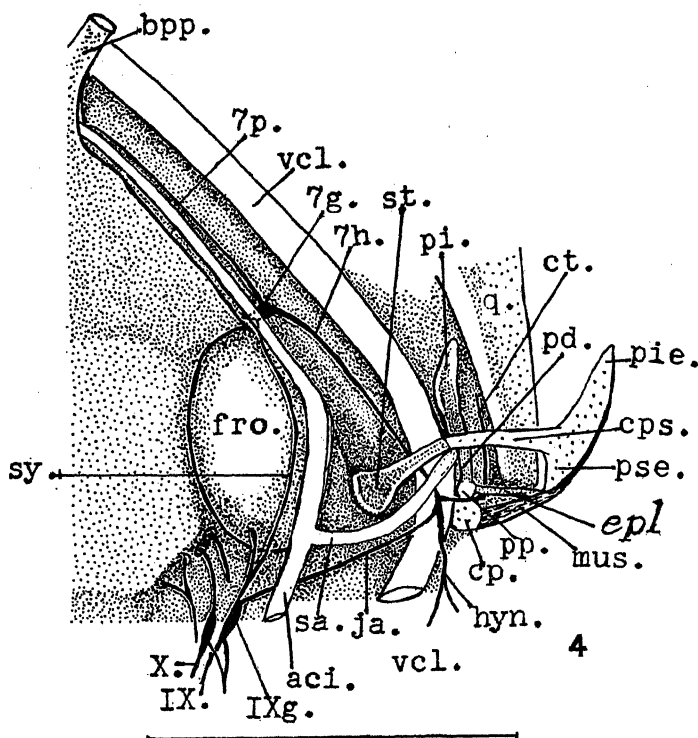
**The Intracapsular Cavity.**—The cavity of the auditory capsule shows the same features as that of *Lacerta* (Gaupp, 1900) or *Eumeces* (Rice, 1920). The main cavity is divisible into an anterior smaller cavum vestibulare anterior (Text-fig. 15A) and a larger posterior cavum vestibulare posterior, the two being separated by a septum intervestibulare (*si*). This septum shows two orifices normally in lizards, viz. a posterior median which permits the utriculus lying in the cavum vestibulare posterior to communicate with the recessus utriculus in the cavum vestibulare anterior and an anterior lateral foramen which is closed by a membrane. In *Calotes*, however, the median one is not an orifice since it is not limited by cartilage, while the lateral is normal.

The recessus ampullae of the anterior (*raa*) and lateral (*ral*) semicircular canals are at different levels from the utricular recess (*ru*) in the cavum vestibulare anterior and lead into the anterior orifices of the canals respectively (in the figure only one is seen, *aoa*). The roof of the cavum vestibulare anterior is, therefore, formed by the septum semicirculare anterior (*sasc*) till the latter separates itself from the cavum to run dorsally to the gap referred to above (Text-fig. 15C).

The cavum vestibulare posterior is a spacious chamber into which the cavum cochleare (*cc*) opens anterodorsally. Postero-ventrally, the cavum vestibulare posterior gives rise to the recessus ampullae posterior (*rap*) which continues into the inferior opening of the posterior semicircular canal (Text-fig. 15C, *ipsc*). The posterior opening (Text-fig. 15C, *opl*) of the lateral semicircular canal (*lsc*) in the posterior region of the cavum vestibulare

posterius is above the posterior ampullary recess near the septum of the posterior semicircular canal (*spsc*). On the posterior aspect of the septum semicirculare posterius (Text-fig. 15 D, *spsc*) the superior orifice of the posterior semicircular canal (*osp*) and the foramen pro sinu superior (*fps*) are noticed. In the recessus sinus superior (Text-fig. 15 E, *rss*) the posterior orifice (*opa*) of the anterior semicircular canal is noticed and in this region the posterior and anterior semicircular canals merge into the sinus superior.

A brief reference may be made to the cartilaginous canals. The three semicircular canals are separated from the cavum vestibulare by septa; the septum of the anterior canal is horizontal (Text-fig. 15 A, *sasc*) and forms the roof of the cavum vestibulare anterius as in *Lacerta*, but slightly anterior to the region of the posterior opening of the lateral semicircular canal, the otic capsule wall (Text-fig. 15 C, *slsc*) forms the roof of the cavum vestibulare posterius, the membranous anterior semicircular being enclosed in its own canal (*asc*) and thus being separated from the otic capsule by a gap. Posteriorly, however, at the region of the sinus superior, the semicircular canal (Text-fig. 15 E, *asc*) again joins the cavum vestibulare posterius to open into the recessus sinus superior (*rss*). The septum of the lateral semicircular canal (Text-figs. 15 A, 15 B, *slsc*) is obliquely vertical and longitudinal; it partly forms the inner wall of the cavum vestibulare posterius. It anteriorly meets the septum intervestibulare (Text-fig. 15 A, *si*) on the lateral side and projects into the posterior part of the cavum vestibulare anterius. The posterior extension of the septum (Text-fig. 15 C, *slsc*) is seen in the region where the lateral semicircular canal opens into the cavum vestibulare posterius by its posterior orifice (*opl*). The septum of the posterior semicircular canal is short (Text-figs. 15 C, 15 D, *spsc*), vertical and transverse and forms the posterior inner wall of cavum vestibulare posterius. The anterior face of this septum limits the posterior opening of the lateral semicircular canal, while its posterior faces the sinus superior. In this posterior semicircular septum is the foramen pro sinu superior (Text-figs. 15 C, 15 D, *fps*), one edge of which is limited by the otic capsule wall. At the region where the lateral



TEXT-FIG. 4.

The region of the columella auris of a 8.0-mm. (H.-L.) young of *Calotes versicolor* (Daud.) to show the relation of blood-vessels and nerves; *aci*, arteria carotis interna; *bpp*, basipterygoid process; *cp*, crista parotica; *cps*, cartilaginous connexion between stapes and the insertion plate of columella; *ct*, chorda tympani; *epl*, the extrapleural-parotic (processus) ligament; *fro*, foramen rotundum; *hf*, hypoglossal foramina; *hyn*, hypoglossal nerve; *ja*, Jacobson's anastomosis; *mus*, part of stylomastoid muscle spanning the extrapleural and crista parotica; *pd*, ligamentary processus dorsalis; *pi*, cartilaginous processus internus; *pie*, pars inferior of insertion plate; *pp*, processus paroticus; *pse*, pars superior of insertion plate; *q*, quadrate; *sa*, stapedial artery; *st*, stapes; *vcl*, vena capitis lateralis; *7g*, facial ganglion; *7h*, hyomandibular branch of facial nerve; *7p*, palatinus facialis; *IX*, glossopharyngeal nerve; *IXg*, ganglion on the IX; *X*, vagus nerve.

semicircular canal enters the cavum vestibulare posterius there is a short projection of cartilage (Text-fig. 15 c, *ise*) separating the inferior orifice of the posterior (*ipsc*) and the posterior opening of the lateral semicircular canals (*opl*).

The foramen perilymphaticum noticed in the posterior wall of the cavum cochleare faces into the fissura metotica. Like *Lacerta*, the recessus scalae tympani of *Calotes* shows the same arrangement: the recessus scalae tympani medialis (covered over by connective tissue) and the recessus scalae tympani lateralis (covered over by secondary tympanic membrane) and the IX nerve pierce both to gain exit.

The Columella Auris.—In the fenestra vestibuli (Text-fig. 11; Text-fig. 13 A, *fo*) fits in the footplate or stapes (*st*) of the columella auris, a product of the visceral arch. The insertion plate (Text-fig. 11; Text-figs. 16 B, 16 C, *ip*) which fits into the tympanic membrane is connected with the stapes by a cartilaginous bar (the whole being called extracolumella) and shows the following projections: a long cartilaginous process directed anteroventrally—the pars inferior (Text-fig. 13 A; Text-fig. 4, *pie*) and the part of the plate which is postero-dorsal to the connecting piece of cartilage (Text-figs. 11, 13 A, 16 B; Text-figs. 4, 5, *cps*)—the pars superior (Text-fig. 13 A; Text-fig. 4, *pse*). From the pars superior there are two short cartilaginous projections: one directed towards the quadrate—the processes accessorius anterior (Text-fig. 5, *paa*) from which a thin ligament arises and is inserted to the quadrate, and a posterior—the processus accessorius posterior (*pap*). From this posterior process, no connexion either cartilaginous or ligamentary is noticed with the ceratohyal, unlike what is described by Fuchs (1907), Kunkel (1912), and Shiino (1914) in various reptiles. In addition, starting from the pars inferior and passing over the pars superior there is a thick ligament (Text-fig. 4, *epl*) which meets the processus paroticus (*pp*) at the region where the processus dorsalis (*pd*) (see below) unites with the former. The chorda tympani (*ct*) passes dorsolaterally to this ligament. There is also a muscle (*mus*) arising from the insertion plate (from the pars superior region) and getting inserted to the crista parotica (*cp*) along with the stylohyoid

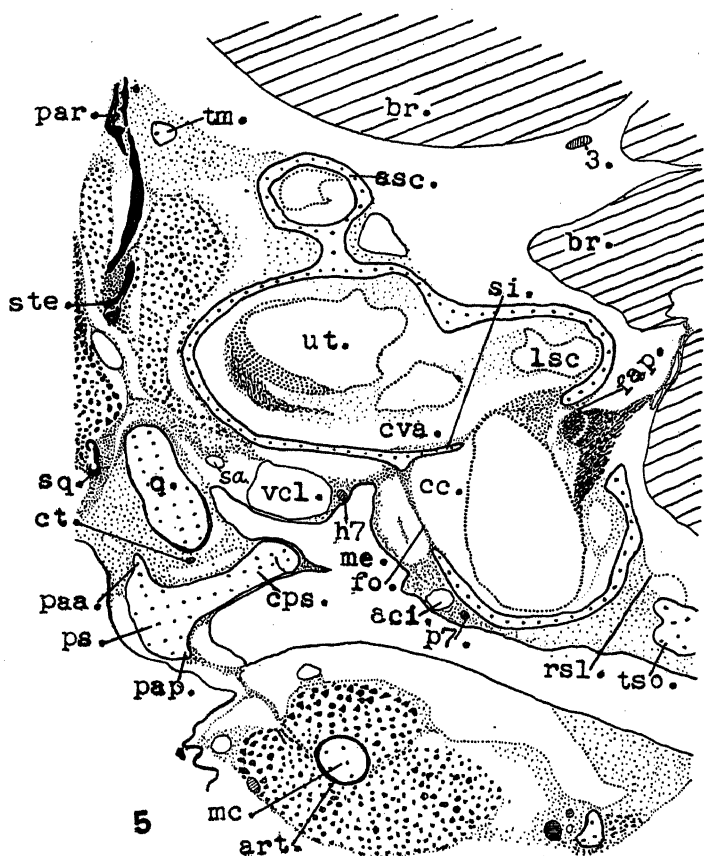


muscle. Such a muscle is also noticed by Brock (1932) in *Lygodactylus*.

No reference is made by Gaupp (1900) to these ligamentary connexions. Rice (1920) described in *Eumeces* a parotic ligamentary connexion (with the chorda tympani passing laterally to this) and Fuchs described a connexion between the processus accessorius anterior and the quadrate. Versluys (1903) described a ligamentary connexion between insertion plate and parotic process in *Pachydactylus*; in *Lygodactylus* (Brock, 1932) a transient cartilaginous connexion with the parotic process with which the ceratohyal also united is noted. In *Agama* (Brock, 1932) and *Varanus* (Bahl, 1937) the parotic ligamentary connexion is described, and Versluys (1903) in his schematic figure of the columella auris for lizards shows a ligament arising from the pars inferior (as in *Varanus*, Bahl, 1937) and reaching the processus paroticus or intercalary. In *Amphisbaena* Versluys (1898) mentioned the absence of this connexion.

From the connecting cartilaginous rod between insertion plate and stapes arises the cartilaginous processus internus (Text-fig. 13A; Text-fig. 4, *pi*) in *Calotes* and projects anteroventrally towards the quadrate with which it articulates as in *Lacerta* (Gaupp, 1900) and *Agama* (Brock, 1932). In *Eumeces* (Rice, 1920) it does not reach the quadrate. In *Lygodactylus* (Brock, 1932) and adult *Chalcides* (Haas, 1936) it is absent. According to Versluys (1898) the adult *Scincidae* lack it; in other lizards like *Uroplates*, *Anguidae* and embryos and adults of *Geckonidae* it is also absent (Versluys, 1903).

There is another ligamentary connexion in *Calotes*—the processus dorsalis (Text-figs. 11, 13A, 16B; Text-fig. 4, *pd*) uniting the connecting cartilaginous piece of the columella (*cps*) with the processus paroticus (*pp*). It arises dorsally from the columella at the region where the processus internus is given off ventrally. Versluys (1903) described, in this connexion, how the dorsal portion of the original cartilaginous processus dorsalis formed the intercalary or processus paroticus when it met the crista parotica, while the connexion between the intercalary and columella remained as a ligament.



TEXT-FIG. 5.

Transverse section of a 8.0-mm. (H.-L.) young of *Calotes versicolor* (Daud.) in the region of middle ear,  $\times 55$ . *aci*, arteria carotis interna; *art*, articular; *asc*, anterior semicircular canal; *br*, brain; *cc*, cavum cochlearis; *cps*, connecting cartilage between stapes and insertion plate; *ct*, chorda tympani; *cva*, cavum vestibulare anterius; *fap*, foramen acousticum posterius; *fo*, foramen vestibuli; *h7*, hyomandibularis facialis; *lsc*, lateral semicircular canal; *mc*, Meckel's cartilage; *me*, middle ear; *paa*, processus accessorius anterior; *pap*, processus accessorius posterior; *par*, parietal; *ps*, pars superior of insertion plate; *p7*, palatinus facialis; *q*, quadrate; *rsl*, recessus scalae tympani lateralis; *sa*, stapedia artery; *si*, septum intervestibulare; *sq*, squamosal; *ste*, supratemporal; *tm*, taenia marginalis; *tso*, tuberculum spheno-occipitale; *ut*, utriculus; *vcl*, vena capitis lateralis; *3*, oculomotor nerve.

The processus dorsalis is absent in *Eumeces* (Rice, 1920), *Lygodactylus* (Brock, 1932), and adult *Chalcides* (Haas, 1936). In *Varanus* (Bahl, 1937) also a processus dorsalis is said to be absent; however, a ligamentary connexion in exactly the same position as the processus dorsalis is drawn (p. 159, Text-fig. 13); but the chorda tympani bears no relation to this. Having examined *Varanus*, I am inclined to consider the ligamentary connexion as a processus dorsalis, though the chorda tympani does not surround it from backwards.

The branches of the hyomandibular nerve (Text-fig. 4, *7h*, *7p*, *ct*), the jugular vein, and the stapedia artery (*sa*) bear typical relations with the processus dorsalis (*pd*), processus internus (*pi*), and the ligamentary pars superior-parotic connexion as described in *Lacerta* (de Beer, 1937).

#### 4. The Orbitotemporal Region.

As in other lizards, the orbitotemporal region is characterized by the absence of a roof. The trabeculae (Text-fig. 12, *tr*) running anteriorly from the basal plate unite anteriorly to the hypophysial fenestra (*hyf*) forming the trabecula communis (*tc*). In *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920) the trabecula communis runs anteriorly under the interorbital septum. In *Calotes* it stops short at the anterior end of the hypophysial fenestra. In *Chelone* Fuchs (1912) stated that the trabeculae did not enter into the formation of the interorbital septum.

The internal carotid artery becomes intracranial through the hypophysial fenestra.

The basiptyergoid articulation is noticed as in *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920) between the basitrabecular process of the trabecula (Text-fig. 11, *btp*) and the broad meniscus pterygoideus cartilage (*mp*), which represents the basal process of the pterygoquadrate. No connexion between the meniscus cartilage and the base of the processus ascendens (*pas*) is noticed in any of the stages of *Calotes* studied.

The interorbital septum (Text-fig. 11, *ios*) makes a sharp bend near the olfactory region. Near the olfactory capsule, the sep-

tum shows an anterior smaller (*f1*) and a posterior larger (*f2*) septal fenestra. Connecting the interorbital septum anteriorly and forming the anterior boundary of this large posterior fenestra there is the round bar of cartilage—the united preoptic pillar (*pop*) uniting posterodorsally with the planum supraseptale (*pls*). From the ventral aspect of the posterior portion of the planum supraseptale there descends a vertical pillar (*plos*) which meets the interorbital septum below and forms the posterior boundary of the large fenestra (*f2*).

In *Lacerta* (Gaupp, 1900), in the region corresponding to the preoptic pillar of *Calotes*, the anterior portion of the supra-septal cartilage is broad with a median depression; however, from the anterior portion of the planum there are two cartilaginous projections, one on each side, as in *Calotes* (Text-figs. 11, 12, *apls*). These two projections are just indicated in *Eumeces* (Rice, 1920). From the posterodorsal edge of the planum there arises in *Lacerta* the taenia marginalis, which, however, is wanting in *Calotes*. A small projection from the lateral portion of the planum represents, in *Calotes*, the anterior end of the taenia marginalis (*atm*); similarly a small projection from the anterior margin of the otic capsule represents the posterior reminiscence of this (*ptm*). In *Lacerta* (Gaupp, 1900) the taenia medialis arising from the posteroventral edge of the planum supraseptale meets and fuses with the vertical pila antotica; it is also connected with two more pillars—the pila accessoria (connecting with taenia marginalis) and pila metoptica (uniting and forming the subiculum infundibuli and cartilago hypochiasmatica). In *Calotes* since there is no taenia marginalis, there is no pila accessoria; the taeniae mediales (*tme*) progress towards the hypophysial fenestra and fuse. Slightly anteriorly to this fusion the taeniae are met by a short round obliquely vertical pillar (*chsp*) from the interorbital septum—the united cartilago hypochiasmatica, subiculum infundibuli, and pila metoptica. In *Lacerta* (de Beer, 1937) that part of the taenia in front of the pila accessoria is called taenia medialis, while that behind it is the metoptic pila. In *Calotes*, such a differentiation is not possible. The taenia posterior to this median cartilaginous pillar represents

the reminiscence of the connexion of the taenia with the pila antotica.

The various foramina in this region may now be recounted:

(i) the two septal fenestra (Text-fig. 11, *f1*, *f2*) anteriorly do not transmit anything.

(ii) the optic fenestra (*of*) is bounded anteriorly by the vertical pillar (*plos*) of the interorbital septum; below by the united cartilago hypochiasmatica, subiculum infundibuli, pilae metopticae and interorbital septum, and dorsally by the taenia medialis.

(iii) the metoptic fenestra (*fm*) is bounded anteriorly by the cartilago hypochiasmatica (plus subiculum infundibuli and pilae metopticae), above by the posterior part of the taenia medialis, below by the trabecula, and posteriorly there is no definite boundary. The third and fourth cranial nerves take exit in this fenestra.

(iv) the prootic fenestra (in front of incisura prootica, *ipr*) is no definite fenestra; it may be said that the pila antotica (*pa*) and processus ascendens (*pas*) bound it anteriorly, while below it is bounded by the basal plate and behind by the auditory capsule and prefacial commissure (*pfc*). Dorsally it is not limited in *Calotes*, while in *Lacerta* (Gaupp, 1900) the taenia marginalis roofs it. It must, however, be noted that the trigeminal ganglion is lodged in an extracranial space, the cavum epiptericum, which is slightly posterior to the processus ascendens in the incisura externally to the skull wall.

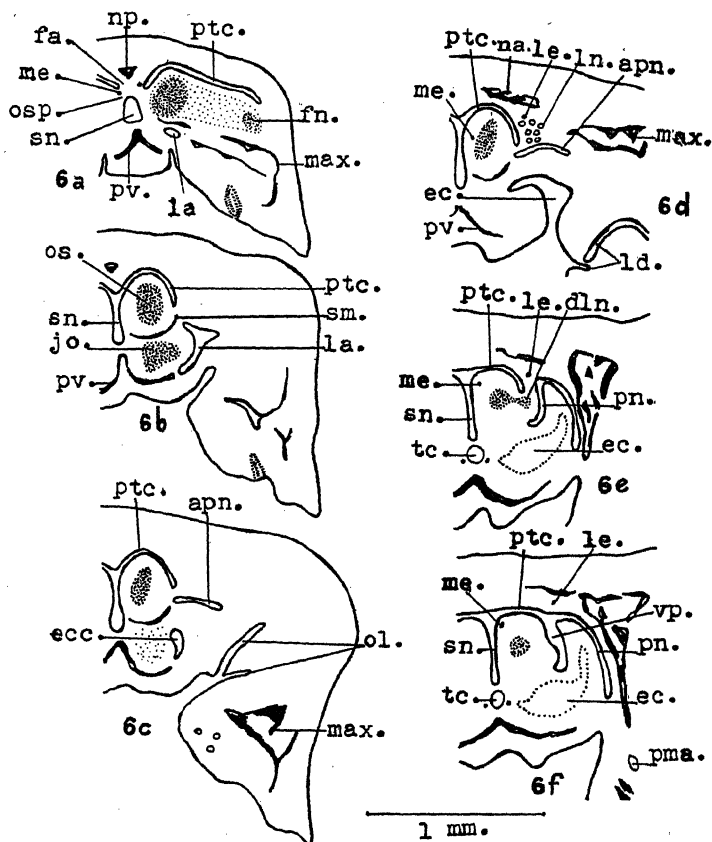
A sclerotic cartilage is well developed.

## 5. The Ethmoid Region.

Since the topography of the cartilages in the nasal region differs considerably from the descriptions of *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920), I propose to describe in detail the olfactory region of *Calotes*.

The anterior sections of the nasal capsule in *Calotes* reveal that the fenestra narina (Text-fig. 14c, *an*) is lateral and this part of the olfactory sac, the cupola anterior (*ca*), is covered over by the anterior parietotectal cartilage. In *Lacerta*

(Gaupp, 1900) the median branch of the ethmoid nerve gets exit by the foramen apicalis situated one on each side in the cupola anterior; the fenestra superior is above and posterior to each apical foramen. In *Calotes* there is a gap between the nasal septum and the parietotectal cartilage (Text-fig. 14 A; Text-fig. 6 A, *fa*) and through this, the combined fenestrae apicales, the ramuli medialis (*me*) come out. A fenestra superior is absent in *Calotes*. Supporting Jacobson's organ, the lamina transversalis anterior (Text-figs. 14 B, 14 C, *la*) is noticed with its inner limb united (*clsn*) with the unfenestrated septum nasi (*sn*). A few sections posteriorly where Jacobson's organ is cut, it is seen as a discrete cartilage (Text-fig. 6 B, *la*); no projection of it enters into the floor of Jacobson's organ as in *Ablepharus* and *Chalcides* (Haas, 1935, 1936). In *Calotes* it divides into two, one partially embracing Jacobson's organ (Text-figs. 14 B, 14 C; Text-fig. 6 C, *ecc*) and the other (*apn*) situated dorsally to this but laterally to the parietotectal cartilage; this latter is an anterior extension of the paranasal cartilage. In *Lacerta*, the lamina transversalis anterior not only forms a support for Jacobson's organ but also a complete ring of cartilage (zona annularis) round it; in *Calotes* since the side wall is incomplete in this region there is no ring formation. Further from the posteromedian part of the lamina transversalis anterior there arises in *Lacerta* (Gaupp, 1900) the paraseptal cartilage (free from the nasal septum). It unites with the anterior portion of lamina orbitonasalis; in *Calotes* there is no paraseptal cartilage at all in this region. However, there is the process corresponding to the ectochoanal cartilage (*ecc*). The nasal glands appear (Text-fig. 6 D, *ln*) in the gap between the parietotectal (*ptc*) and the anterior portion of paranasal cartilage (*apn*); the ramus lateralis (*le*) of the ethmoid nerve is associated with these glands. This region would, therefore, correspond with the cavum conchale of *Lacerta*. The paranasal cartilage is unconnected with the parietotectal in this region nor does it form a concha nasalis by reduplication as in *Lacerta* and *Eumeces*. The lateral nasal glands are, therefore, not at all enclosed. In Text-fig. 6 E, the opening of the duct (*dln*) from these glands into the olfactory sac is noticed.



TEXT-FIGS. 6A-6F.

Consecutive transverse sections in the region of nasal capsule of 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.). *apn.*, anterior part of paranasal cartilage; *dln.*, duct entering cavum nasale principale from lateral nasal glands; *ec.*, recessus ectochoanalis; *ecc.*, ectochoanal cartilage; *fa.*, conjoint fenestrae apicalis; *fn.*, fenestra narina; *jo.*, Jacobson's organ; *la.*, lamina transversalis anterior; *ld.*, ductus nasolachrymalis; *le.*, lateral ethmoid nerve; *ln.*, lateral nasal glands; *max.*, maxilla; *me.*, median ethmoid nerve; *na.*, nasal; *np.*, nasal process of premaxilla; *ol.*, opening of nasolachrymal ducts; *os.*, cavum nasale principale; *osp.*, orifice between septum nasale and parietotectal cartilage; *pma.*, processus maxillaris anterior; *pn.*, paranasal cartilage; *ptc.*, parietotectal cartilage; *pv.*, prevomer; *sm.*, septomaxilla; *sn.*, septum nasi; *tc.*, trabecula communis; *vp.*, vertical pillar.

However, in the 8.0 mm. young, the opening of this duct is slightly posterior to the vertical cartilage formed by the union of the paranasal and parietotectal cartilages. In this region (Text-fig. 6 E) the cavum extraconchale (*ec*) is also seen and this is embraced by the paranasal cartilage (*pn*) having assumed the shape of a horseshoe. The lateral ethmoid nerve (*le*), as in the previous figure, is external to the parietotectal cartilage (*ptc*). Posteriorly (Text-fig. 6 F) the parietotectal and paranasal cartilages unite forming a vertical pillar (*vp*) and still the ramus lateralis (*le*) is external to the cartilage. This pillar separating the olfactory from the extraconchal recess does not represent the fused posterior walls of the conchae nasales since the auditus concha in all the Lacertilia is posterior to the epiphaneal foramen; also it does not represent the crista semicircularis of mammals since that is also posterior to the epiphaneal foramen. The paranasal cartilage continues posteriorly into the lamina orbitonasalis (Text-figs. 14 A, 14 C, *lon*), the demarcating orifice being the epiphaneal between the two.

The epiphaneal foramen (Text-fig. 14 A, *fe*) is peculiarly disposed. The ramus lateralis nasi situated ventrally to the commissura spheno-ethmoidalis (*cse*) becomes intracranial when the latter unites with the lamina orbitonasalis (*lon*) and gets exit a few sections anteriorly. In *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920) the ramus lateralis nasi gets out through the epiphaneal foramen and enters the cavum conchale by its anterior opening, the auditus concha.

The olfactory nerve running dorsomedially to the sphenethmoid commissure enters the fenestra olfactorium advehens by getting out through the foramen olfactoria evehens; this is exactly as in *Lacerta* (de Beer, 1937). The ethmoid nerve (ophthalmicus profundus V) enters the extracranial orbitonasal space (cavum orbitonasale) ventrally to the sphenethmoid commissure in *Calotes* and before entering the olfactory sac divides into two; the ramulus medialis enters by its own orifice in the cartilaginous wall and not by the advehent opening as in *Lacerta* and gets out through the conjoint apical orifice in *Calotes*. The passage of the lateral ramulus has been described above.



The width of the nasal capsule in the posterior region could only be ascribed to the formation of an extracranial portion since a concha nasalis is not formed in *Calotes*.

The hinder part of the nasal capsule is free from the nasal septum in *Lacerta*; it is also free ventrally in *Calotes* (Text-fig. 14 B) but attached to nasal septum dorsally by means of the lamina orbitonasalis. The free olfactory floor in the posterior region formed by the lamina orbitonasalis shows a projection by the side of the nasal septum. This projection represents the reminiscence of the paranasal connexion (*pps*).

The lamina orbitonasalis shows two projections at different levels. The anterior one at about the level of the vertical pillar referred to previously represents the processus maxillaris anterior (Text-figs. 14 B, 14 c; Text-fig. 6 F, *pma*). Arising from the posterior wall of the lamina orbitonasalis on either side of the interorbital septum (*ios*) there is a short projection which I have called the posterior laminal process (*plp*). This does not represent the posterior maxillary process, since in the 8.0-mm. stage this is noticed to arise from the posterior aspect of the lamina orbitonasalis and the posterior laminal process is also present.

## 6. The Pterygoquadrate.

The pterygoquadrate, it has already been pointed out, was noticed as dense mesenchyme in the 3.6-mm. stage extending anteriorly from the region where the future basitrabecular articulation would be formed. The next and subsequent stages show that an anterior pterygoid process is not developed and the processus maxillaris posterior of the lamina orbitonasalis represents the remnants of the ethmoid connexions of the pterygoquadrate. A processus ascendens is well formed (Text-fig. 11; Text-fig. 12, *pas*) and does not show any connexion dorsally with the otic capsule as in *Platydictylus* (Hafferl, 1921). In adult *Varanus* (Bahl, 1937) the dorsal part of the processus ascendens is united with the otic capsule. Narayanaswamy Iyer (1943) also described a dorsal cartilaginous otic-ascendens connexion in *Calotes*. There is a meniscus pterygoideus (Text-fig. 11, *mp*) cartilage between the ventral portion

of the processus ascendens and the basitrabecular projection (*btp*), representing the basal process of the pterygoquadrate.

The otic articulation of the pterygoquadrate is noticed as in other lizards (*Lacerta* Gaupp, 1900; *Eumeces* Rice, 1920); the dorsal portion of the quadrate part of the pterygoquadrate (Text-fig. 11, *oa*) articulates with the crista parotica (*cp*) and the processus paroticus (*pp*).

When the quadrate becomes ossified, the anterior crescentic portion of it forms an 'auditory cup' for the attachment of the anterior margin of the tympanic membrane.

The two rami of Meckel's cartilage (Text-fig. 7, *mc*) are almost straight circular rods united at the tip (*sm*), while posterior to the region of articulation with the quadrate (*aq*) is the processus retroarticularis (*pra*). It is interesting to note that, as in other lizards, Meckel's cartilage chondrifies much earlier than the neurocranium.

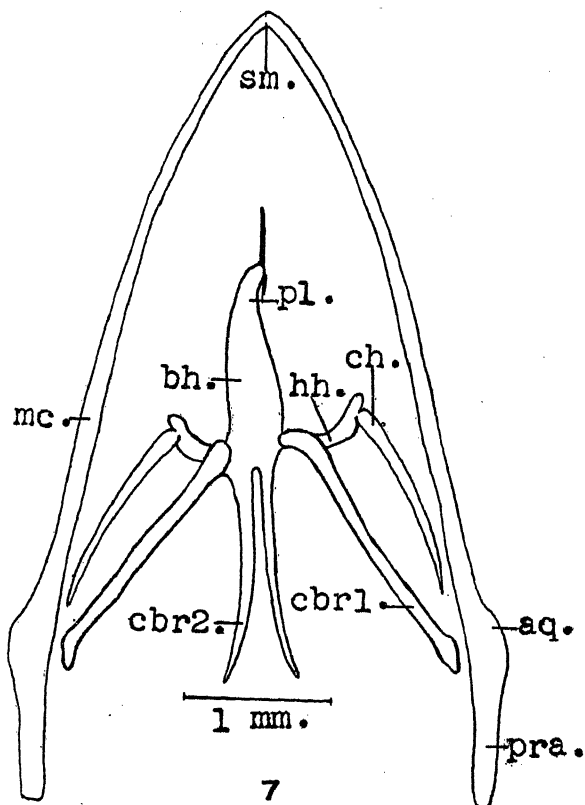
## 7. The Hyoid Apparatus.

The dorsal part of the hyoid arch, viz. the columella auris, was described in connexion with the otic capsule. The basihyal (Text-fig. 7, *bh*) gives rise anteriorly to a processus lingualis or entoglossus (*pl*), which enters the tongue, and to two lateral processes, one from each side, the hypohyal (*hh*). From each hypohyal (*hh*) extends the ceratohyal (*ch*) of its side. As already said, there is no connexion between the processus accessorius posterior of the columella auris and the ceratohyal.

From the basihyal there project posteriorly, the first (*cbr1*) and second (*cbr2*) ceratobranchials; ceratobranchial 1 is articulated with the basihyal by a ball and socket arrangement (Gnanamuthu, 1937; Narayanaswamy Iyer, 1943). Unlike *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920) the ceratobranchial 2 is a complete rod of cartilage and not observed to be in two parts.

## II. Osteocranium of a just-hatched young of *Calotes versicolor* (Daud.), H.-L. 8.0 mm.

I am describing the cranial bones in a just-hatched young of *Calotes* with a head-length of 8.0 mm. As already



TEXT-FIG. 7.

Meckel's cartilage and hyobranchial apparatus of a 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.). *aq.*, articular facet for quadrate; *bh.*, basihyal; *cbr1*, ceratobranchial 1; *cbr2*, ceratobranchial 2; *ch.*, ceratohyal; *hh.*, hypohyal; *mc.*, Meckel's cartilage; *pl.*, processus lingualis; *pra.*, processus retroarticularis; *sm.*, symphysis meckelii.

said, the adult skull has been described by Narayanaswamy Iyer (1943).

#### Membrane bones.

The nasal (Text-fig. 16 A, *na*) starts on each side of the nasal process of the premaxilla (*pmx*) posterior to the conjoint apical

foramen. It is disposed on the parietotectal cartilage and extends as far as the epiphaneal foramen. In the adult, also, it is paired (Narayanaswamy Iyer, 1943). It is unpaired in *Varanus* (Bahl, 1937).

The frontal (*fr*) arises over the lamina orbitonasalis posterior to the epiphaneal foramen, medially to the prefrontals (*prf*) and dorsolaterally to the sphenethmoid commissure. The two bones are disposed near each other on the preoptic pillar (Text-fig. 16 c, *pop*), lateral to the supraseptal (*ps*), and end postoptically diverging from each other. In the adult (Narayanaswamy Iyer, 1943) the two frontals unite to form a single bone and the gap between the two bones in this stage is bridged by osseous tissue.

Each parietal (Text-figs. 16 A, 16 c, *par*) arises medially at the posterior end of the diverging frontal and the cranial roof between the two parietals is bridged by connective tissue; in the region of the anterior semicircular canal, the reminiscent taenia marginalis projection from the otic capsule is dorsal and widely separated from the parietal and the bone ends on the crista parotica (*cp*). In *Lacerta* (de Beer, 1937) the frontals and parietals are associated with the taenia marginalis, but in *Calotes*, as already noted, this cartilage is not formed. In the adult *Calotes* (Narayanaswamy Iyer, 1943) the two parietals unite to form a large bone with a median orifice—the pineal orifice. From the parietal there are two posterior paroccipital processes, each coming in contact with the exoccipital and quadrate of its side.

The septomaxilla arises dorsally to the union of the lamina transversalis anterior with the nasal septum and lies dorsally to this cartilage and Jacobson's organ (see Text-fig. 6 B, *sm*). It forms the floor of the olfactory sac (Text-fig. 16 B, *smx*) and disappears where the cavum extraconchale enters the choana. In the adult (Narayanaswamy Iyer, 1943) the septomaxilla is disposed ventrally to the nasal process of the premaxilla and medially to the maxilla, in the olfactory region. In *Chameleon* (Haas, 1937) the septomaxilla is wanting.

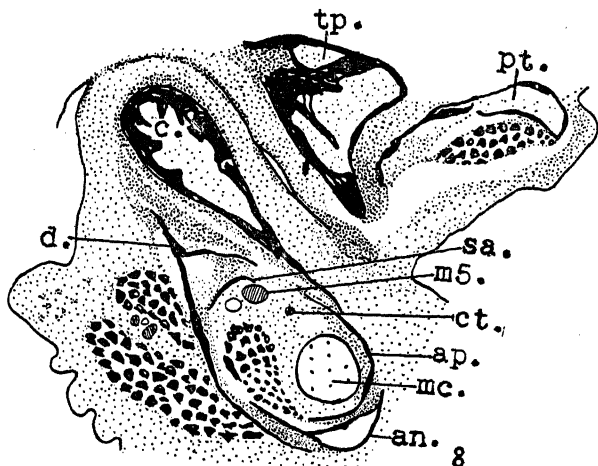
The prefrontal (Text-figs. 16 A, 16 c, *prf*) arises on the posterolateral surface of the nasal capsule, and in the region of the lateral nasal glands they form a lateral boundary for them.

In the region of the lamina orbitonasalis and the sphenethmoid commissures, each prefrontal shows two limbs, a flat and transverse one externally to the frontal and a flat and vertical depending downwards (towards the palatine) and investing the posterolateral face of lamina orbitonasalis. This vertical limb of the prefrontal lies medially to the nasolachrymal opening (Text-fig. 16 c, *pnl*). Between the two vertical limbs of the prefrontals is noticed the orifice which is separated into right and left by the anterior end of the interorbital septum; the two sphenethmoid commissures arise from the dorsal end of this septum and proceed towards the lamina orbitonasalis. The orifice by the side of the nasal septum through which the ethmoid nerve enters the olfactory capsule is the posterior part of the cavum orbitonasale. In the young as well as in the adult (Narayanaswamy Iyer, 1943), since a lachrymal bone is absent, the prefrontal touches the maxilla on the side, and the latter bone, therefore, forms the external boundary of the nasolachrymal orifice.

The prevomer (Text-fig. 16 B, *pv*) appears as a single ossification below the nasal septum and also below the lamina orbitonasalis. Posteriorly where the trabecula communis appears as a separated piece of round cartilage below the nasal septum, the anterior part of palatine appears on each side dorsally to the now separate prevomers and a few sections posteriorly the prevomers disappear.

The palatine (Text-figs. 16 A, 16 B, 16 c, *pal*) arises behind the prevomers and while forming the median boundary of the choana, also gives rise to a maxillary process behind the lamina orbitonasalis. In this region the sections reveal, below the palatine and externally to the palatine and maxilla, two round cartilages; both these are posterior projections from the lamina orbitonasalis running parallel, and the lower one of them represents the processus maxillaris posterior. Similar isolated nodules of cartilage situated one above the other have also been noticed dorsally to the palatine in the processus maxillaris posterior region of *Lacerta* (Gaupp, 1900). The inner border of the palatine in *Calotes* is noticed ventrally to a region where the lamina orbitonasalis gives rise posteriorly to a blunt cartilaginous process.

Extending from the posterior margin of the palatines are the two pterygoids (Text-figs. 16 A, 16 B, 16 C, *pt*). Each pterygoid is flat anteriorly as the palatine and has a ridge ventromedially, and in the region of the ectopterygoid (transpalatine) (Text-fig. 16 B, *ecp*) shows an enlargement. Posterior to the basipterygoid articulation (*bpp*) it becomes dorsoventrally flattened, and



TEXT-FIG. 8.

Transverse section of 8.0-mm. (H.-L.) young of *Calotes versicolor* (Daud.) in the region of the ectopterygoid-ptyergoid-coronoid articulation.  $\times 55$ . *an*, angular; *ap*, articular (with prearticular); *c*, coronoid; *ct*, chorda tympani; *d*, dentary; *mc*, Meckel's cartilage; *m5*, ramus mandibularis V; *pt*, pterygoid; *sa*, supra-angular; *tp*, ectopterygoid (transpalatine).

it ends articulating with the ventral end of the quadrate (*q*). Between the palatine, pterygoid, maxilla, and ectopterygoid is the mediopalatal fossa (*mpf*).

The ectopterygoid (transpalatine) (Text-figs. 16 A, 16 B, *ecp*) spans the jugal-maxilla and the lateral limb of the pterygoid. At the region of articulation with the pterygoid, the coronoid process of the lower jaw (Text-fig. 8, *c*) also enters and the three bones articulate with thick connective tissue in between them.

The premaxilla (Text-figs. 16 A, 16 B, 16 C, *pmx*) is formed by the fusion of the two premaxillae; dorsally there is a large

nasal process, which lies over the nasal septum and extends mesially to the two nasals about half the length of the latter. It carries three teeth and also an anteriorly directed dentinal egg tooth (*et*). Ventrally there is a short median prevomerine projection (Text-fig. 16 B).

On each side of the premaxilla the maxilla (Text-figs. 16 A, 16 B, 16 c, *mx*) forms the outer ventral boundary of the orbit along with the jugal; the anterior boundary of the orbit is formed by the facial process of the maxilla and the prefrontal. The maxilla also sends a process upwards between the prefrontal and nasal. This is the nasal process of the maxilla.

The dentition of the maxilla and premaxilla in the adult *Calotes* is described by Narayanaswamy Iyer (1943).

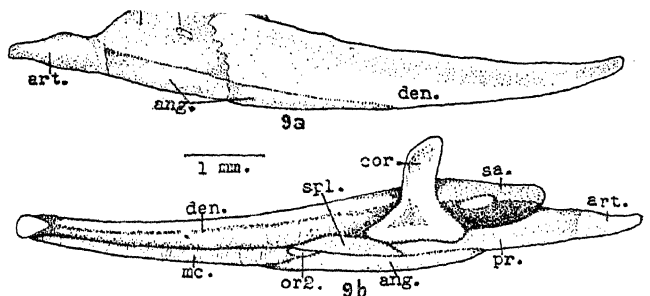
The fenestra narina (Text-figs. 16 A, 16 c, *an*) is lateral and is situated dorsally on the maxilla, but slightly posterior to where the latter meets the premaxilla.

The jugals are broad bones (Text-figs. 16 A, 16 c, *ju*); the anterior extension of each jugal is met with in the anterior orbital region, in association with the maxilla and in the region of the ectopterygoid; the latter articulates with the jugal and maxilla. Posterior to the maxilla, it broadens vertically forming the posterior border of the orbit along with the postfrontal; forming the supratemporal arcade, it ends dorsally to the quadrate where it is separated from the posterior process of the parietal by the squamosal and supratemporal.

The squamosal (Text-figs. 16 A, 16 B, 16 c, *sq*) forms the posterior portion of the supratemporal arcade and ends on the crista parotica (*cp*) externally to the supratemporal.

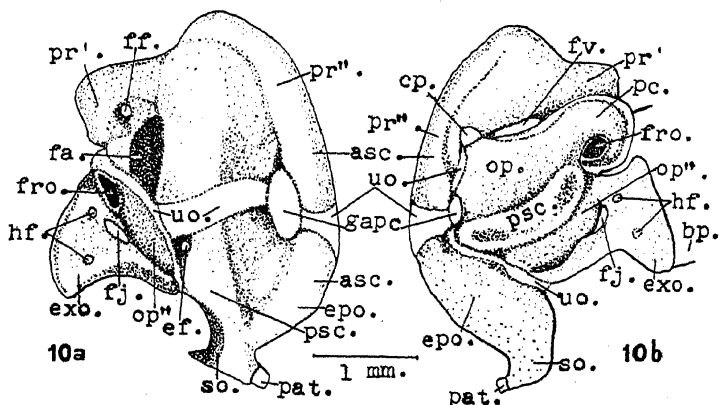
The supratemporal (tabular, Narayanaswamy Iyer, 1943) (Text-figs. 16 A, 16 c, *ste*) arises over the crista parotica internally to the squamosal and runs on the ventrolateral aspect of the posterior process of the parietal. The bone in the adult is not associated with the prootic as in *Varanus* (Bahl, 1937).

The postfrontal (Text-figs. 16 A, 16 B, 16 c, *pfr*) forms along with the lateral supraorbital extension of the frontal and parietal the posterior boundary of the orbit. Each postfrontal has three projections, one dorsal meeting the fronto-parietal



TEXT-FIGS. 9 A, 9 B.

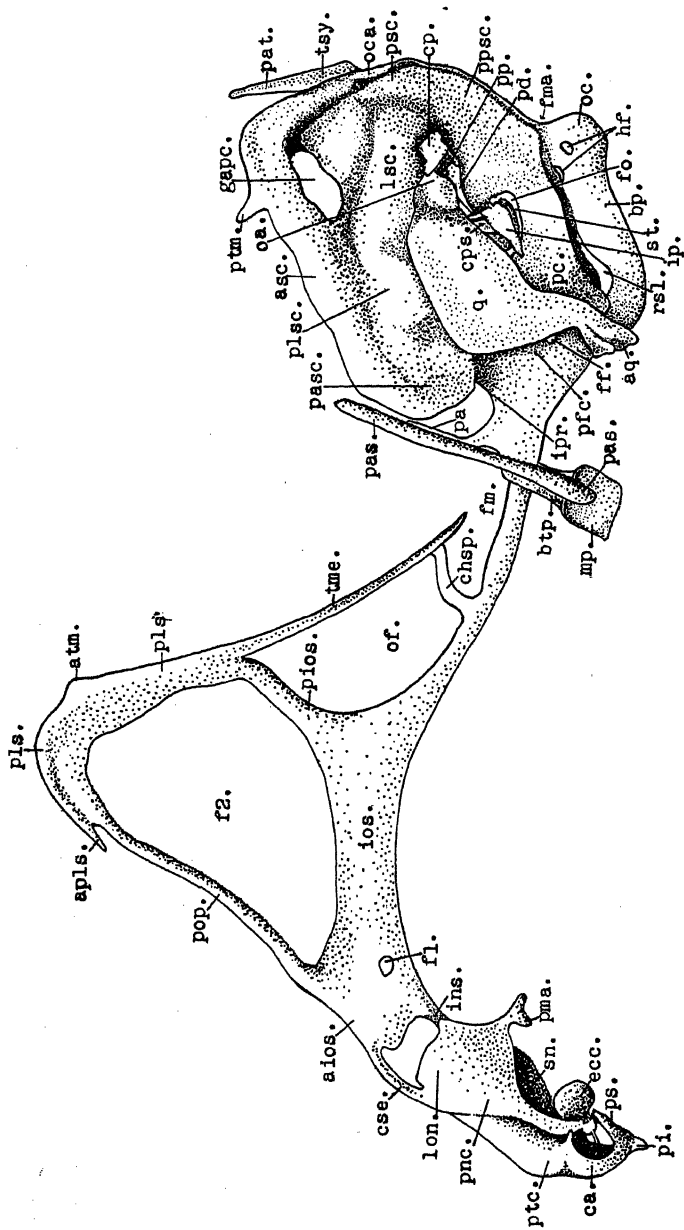
The lower jaw of a 8.0-mm. (H.-L.) young of *Calotes versicolor* (Daud.), 9 A, outer aspect; 9 B, median aspect. *ang.*, angular; *art.*, articular (with prearticular); *cor.*, coronoid; *den.*, dentary; *mc.*, Meckel's cartilage; *or1*, orifice for the lateral cutaneous branch of mandibular V; *or2*, orifice for the main trunk of alveolaris inferior nerve; *pr.*, prearticular part of articular; *sa.*, supraangular; *spl.*, splenial.



TEXT-FIGS. 10 A, 10 B.

Otic capsule of a 8.0-mm. (H.-L.) young of *Calotes versicolor* (Daud.), (alizarin preparation); 10 A, inner view; 10 B, outer view. *asc.*, anterior semicircular canal; *bp.*, basal plate; *cp.*, crista parotica; *ef.*, foramen endolymphaticus; *epo.*, epistotic; *exo.*, exoccipital; *fa.*, foramen acusticum; *ff.*, facial foramen; *fj.*, foramen jugulare; *fro.*, foramen rotundum; *fv.*, fenestra vestibuli; *gapc.*, gap between the anterior and the other semicircular canals; *hf.*, hypoglossal foramina; *op.*, opisthotic in the lateral semicircular canal region; *op''.*, opisthotic in the posterior semicircular canal region; *pat.*, processus anterior tecti; *pc.*, prominentia cochlearis; *pr'*, prootic outside the otic capsule; *pr''.*, prootic in the anterior semicircular canal region; *psc.*, posterior semicircular canal; *so.*, supra-occipital; *uo.*, unossified parts.



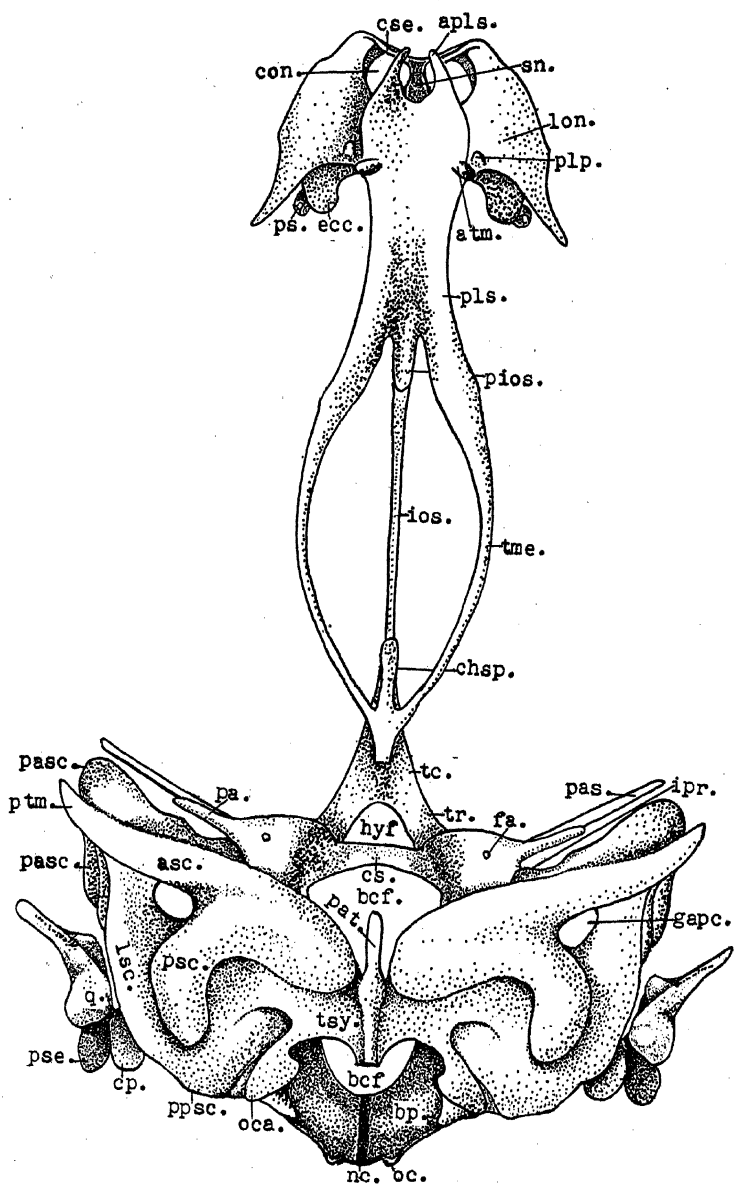


TEXT-FIG. 11.

# TEXT-FIG. 11.

The chondrocranium of a 8.0-mm. (H.L.) embryo of *Calotes versicolor* (Daud.), side view of model,  $\times 35$ ; Meckel's cartilage and the right otic capsule are not included. *aio*s, anterior portion of interorbital septum; *apl*s, anterior projection from the planum supraseptale; *aq*, articular facet for quadrate; *asc*, anterior semicircular canal; *atm*, anterior projection of taenia marginalis; *bp*, basal plate; *btp*, basitrabecular process; *ca*, cupola anterior; *chap*, united cartilago hypochiasmatica, subiculum infundibuli and pilae metopticae; *cp*, crista parotica; *cps*, cartilage connecting stapes with insertion plate; *cse*, commissura sphenothmoidalis; *ecc*, ectochoanal cartilage; *f1*, anterior fenestra in interorbital septum; *f2*, larger fenestra in the same; *ff*, facial foramen; *fm*, fenestra metoptica; *fma*, foramen magnum; *fo*, fenestra vestibuli; *gapc*, gap between the anterior and other semicircular canals; *hf*, hypoglossal foramina; *ins*, region where the interorbital septum continues into the nasal septum; *ios*, interorbital septum; *ip*, insertion plate of extra-colum-

ella; *ipr*, incisura prootica; *lon*, lamina orbitonasalis; *lsc*, lateral semicircular canal; *mp*, meniscus pterygoideus; *oa*, otic articulation of quadrate; *oc*, occipital condyle; *oca*, occipital arch; *of*, optic fenestra; *pa*, pila antotica; *pas*, processus ascendens; *past*, prominentia ampullaris of the anterior semicircular canal; *pat*, processus anterior tecti; *pc*, prominentia cochlearis; *pd*, processus dorsalis; *pfc*, prefacial commissure; *pi*, processus alaris inferior; *pios*, cartilaginous pillar uniting interorbital septum with planum supraseptale; *pls*, planum supraseptale; *plsc*, prominentia ampullaris of the lateral semicircular canal; *pma*, processus maxillaris anterior; *pnc*, paranasal cartilage; *pop*, preoptic pillar; *pp*, processus paroticus; *ppsc*, prominentia ampullaris of the posterior semicircular canal; *ps*, processus alaris superior; *psc*, posterior semicircular canal; *ptc*, parietotectal cartilage; *ptm*, posterior part of taenia marginalis; *q*, quadrate; *rs*, recessus scalae tympani lateralis; *sn*, septum nasi; *st*, stapes; *tme*, taenia medialis; *tsy*, tectum synoticum.



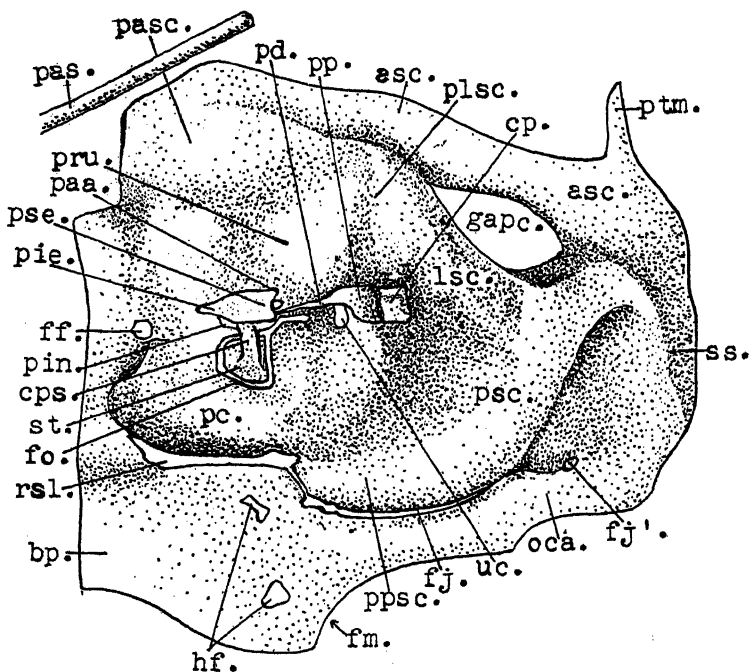
TEXT-FIG. 12.

suture, another ventral articulating with the jugal, while a posterior third not only articulates with the upper margin of the jugal but also with the squamosal; the squamosal articulation is noticed only in the adult condition and not in the young (Text-fig. 16 c). Thus the superior temporal arch is completed anteriorly by this bone.

Though in Agamids a postfrontal is described as absent (Brock, 1932) when the superior temporal arch is formed by the postorbital, I call the bone in the Agamid *Calotes*, a postfrontal. According to Brock, the single bone found in this region of *Pachydactylus* and *Mabuia* is apposed to the frontal and parietal (a criterion according to Camp (1923) of the postfrontal), while in *Agama* it is apposed to the parietal only, and is therefore called the postorbital. Following Camp, 'the postorbital lies between postfrontal, parietal, squamosal and jugal in position ventral and posterior to postfrontal'. Since in *Calotes* the bone abuts against the suture between frontal and parietal, I have called it postfrontal.

#### TEXT-FIG. 12.

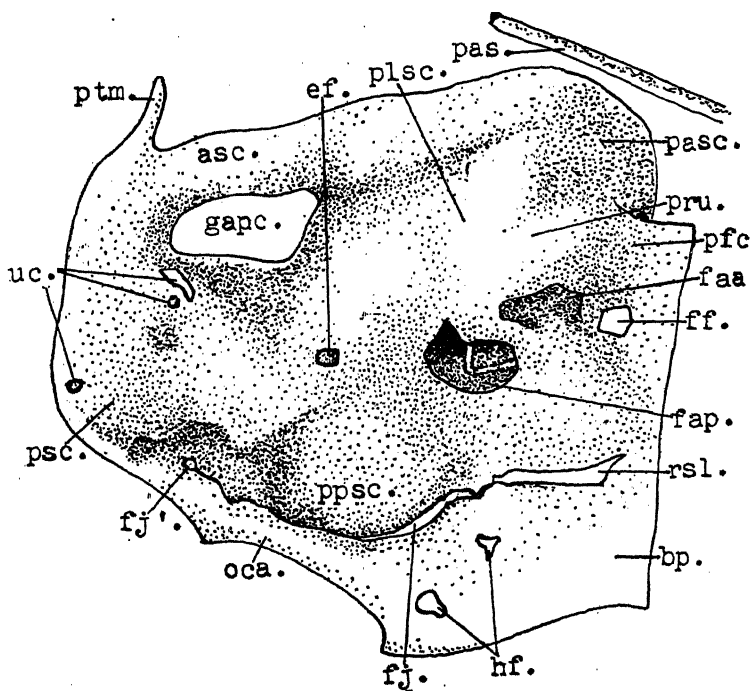
The chondrocranium of a 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.), dorsal view,  $\times 35$ ; the model is so arranged that the planum suprasedale is at right-angles to the otic capsules. *apls*, anterior projection from the planum suprasedale; *asc*, anterior semicircular canal; *atm*, anterior projection of taenia marginalis; *bef*, basicranial fenestra; *bp*, basal plate; *chsp*, united cartilago hypochiasmatica, subiculum infundibuli and pilae metopticae; *con*, cavum orbitonasale; *cp*, crista parotica; *cs*, crista sellaris; *cse*, commissura sphenothmoidalis; *ecc*, ectochordal cartilage; *fa*, foramen abducens; *gapc*, gap between anterior and the other semicircular canals; *hyf*, hypophysial fenestra; *ios*, interorbital septum; *ipr*, incisura prootica; *lon*, lamina orbitonasalis; *lsc*, lateral semicircular canal; *nc*, notochord; *oc*, occipital condyle; *oca*, occipital arch; *pa*, pila antotica; *pas*, processus ascendens; *pasc*, prominentia ampullaris of the anterior semicircular canal; *pat*, processus anterior tecti; *plos*, pillar between the interorbital septum and planum suprasedale; *plp*, posterior laminal process; *pls*, planum suprasedale; *ppsc*, prominentia ampullaris of the posterior semicircular canal; *ps*, processus alaris superior; *psc*, posterior semicircular canal; *pse*, pars superior of extrapleural; *ptm*, posterior part of taenia marginalis; *q*, quadrate; *sn*, septum nasi; *tc*, trabecula communis; *tme*, taenia medialis; *tr*, trabecula; *tsy*, tectum synoticum.



TEXT-FIG. 13 A.

TEXT-FIGS. 13 A AND 13 B.

The otic capsule with adjacent basal plate of a 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 50$ . 13 A, outer view, 13 B, median view of model. *acs*, anterior semicircular canal; *bp*, basal plate; *cp*, crista parotica; *cps*, connecting cartilage between stapes and insertion plate of extracolumella; *ef*, foramen endolymphaticus; *faa*, foramen acusticus anterior; *fap*, foramen acusticus posterior; *ff*, facial foramen; *fj*, foramen jugulare; *fj'*, posterior part of jugular foramen; *fm*, foramen magnum; *fo*, foramen vestibuli; *gapc*, gap between the anterior and the other semicircular canals; *hf*, hypoglossal foramina; *lsc*, lateral semicircular canal; *oca*, occipital arch; *paa*, processus accessorius anterior; *pas*, processus ascendens; *pasc*, prominentia ampullaris of the anterior semicircular canal; *pc*, prominentia cochlearis; *pd*, processus dorsalis; *pfc*, prefacial commissure; *pie*, pars inferior of extrapleural; *pin*, processus internus; *plsc*, prominentia ampullaris of the lateral semicircular canal; *pp*, processus paroticus; *ppsc*, prominentia ampullaris of the posterior semicircular canal; *pru*, prominentia recessus utriculus; *psc*, posterior semicircular canal; *pse*, pars superior of the extra-



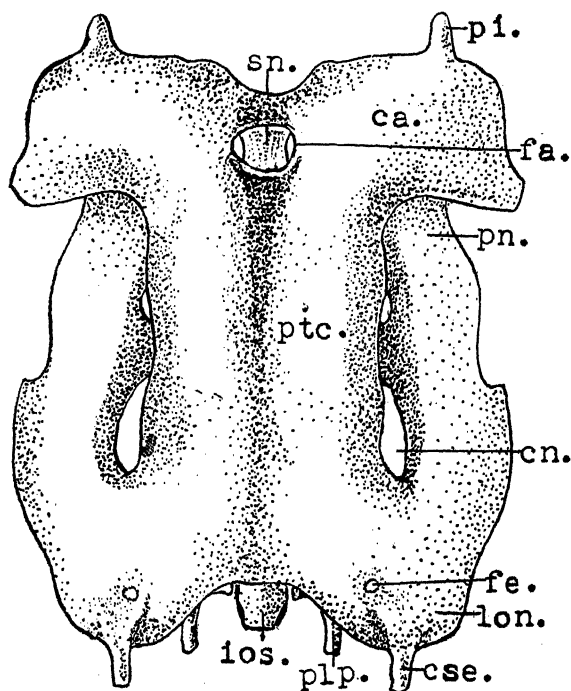
TEXT-FIG. 13 B.

plectral; *ptm*, posterior part of taenia marginalis; *rsl*, recessus scalae tympani lateralis; *ss*, sinus superior; *st*, stapes; *uc*, un-chondrified areas in the otic capsule.

TEXT-FIGS. 14 A-14 C (pp. 274-6).

The olfactory capsule of 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 50$ . 14 A, dorsal view; 14 B, ventral view; 14 C, lateral view. *an*, anterior naris; *apn*, anterior part of paranasal cartilage; *ca*, cupola anterior; *clsn*, union of lamina transversalis anterior and septum nasi; *cn*, cavum conchale; *cse*, commissura sphenoethmoidalis; *ecc*, ectochoanal cartilage; *fa*, united foramina apicalis; *fe*, foramen epiphaniale; *ios*, inter-orbital septum; *la*, lamina transversalis anterior; *lon*, lamina orbitonasalis; *pi*, processus alaris inferior; *plp*, posterior laminal process; *pma*, processus maxillaris anterior; *pn*, paranasal cartilage; *pps*, posterior part of paraseptal cartilage; *ps*, processus alaris superior; *ptc*, parietotectal cartilage; *sn*, septum nasi; *vp*, vertical process.

The parasphenoid underlies the ventral surface of the hypophysial fenestra, leaving anteriorly a small foramen (Text-figs. 16 A, 16 B, *fo*), and runs anteriorly under the interorbital



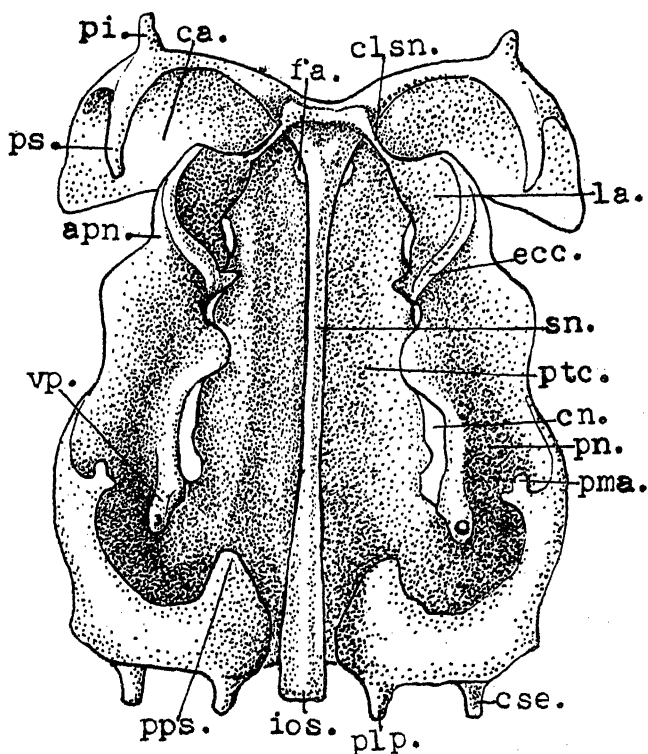
TEXT-FIG. 14 A (see p. 273).

septum (*r*). The median part under the hypophysial fenestra also extends under the basipterygoid processes (*bpp*), while dorsally it is united with the basisphenoid (*bs*). In *Lacerta* (de Beer, 1937) the parasphenoid arises in two lateral and a median centre which unite later. In the adult this bone undergoes fusion with the overlying basisphenoid and the composite bone is called 'sphenoid'.

In each eye-ball of *Calotes* there are twelve sclerotic bones.

A quadratojugal, postorbital, and supraorbitals are absent; also no osteosclerites are seen in *Calotes*.

The prearticular (Text-fig. 9 B, *pr*) arises on the inner aspect of Meckel's cartilage and fuses with the cartilaginous articular (*art*); the chorda tympani which enters posteriorly to the quadrato-meckelian articulation runs between Meckel's cartilage and articular.



TEXT-FIG. 14 B (see p. 273).

The supra-angular (Text-figs. 9 A, 9 B, *sa*) is noticed on the external surface of Meckel's cartilage above the angular; the posterior portion of the dentary covers a portion of the supra-angular as seen in sections (see Text-fig. 8, *d*).

The angular (Text-figs. 9 A, 9 B, *ang*) arises on the ventro-median part of Meckel's cartilage and covers the internal



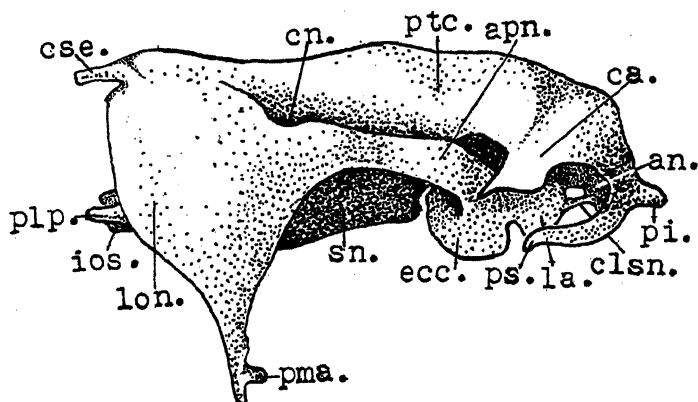
portion of the prearticular, and the upper portion of the angular is also covered over by the dentary (*den*).

The splenial (Text-fig. 9 B, *spl*) arises on the middle portion of Meckel's cartilage medially and is anterior to the angular.

The coronoid (Text-figs. 9 A, 9 B, *cor*) arises dorsomedially to the splenial and forms the anterior limit of the supra-angular.

### Orifices in the Lower Jaw.

1. The orifice in the dorsal aspect of the retro-articular



TEXT-FIG. 14 C (see p. 273).

process posterior to the quadrate articulation for the entry of the chorda tympani is mentioned above.

2. Between the supra-angular and articular portion, medially in front of the quadrate articulation, the ramus mandibularis V enters the lower jaw.

3. Just posterior to the dentary, in a notch in this bone laterally, there is an orifice in the supra-angular for a lateral cutaneous branch of mandibular V (Text-fig. 9 A, *or1*).

4. Anterior to the splenial there is an orifice for the passage of the main trunk of the alveolaris inferior (Text-fig. 9 B, *or2*).

5. A number of small orifices in the lateral aspect of the dentary for smaller branches of the alveolaris inferior are noticed.

### The Cartilage Bones.

The basioccipital (Text-fig. 16 B, *bo*) appears as dorsal and ventral lamellae in the posterior region of the basal plate; the ventral lamella extends and there is a gap between it and the parasphenoid (Text-fig. 16 B, *pas*) anteriorly. The notochord is still seen to be wedged in the basal plate. The cartilaginous process (tuberculum spheno-occipitale) arising from the ventral aspect of the basal plate (Text-fig. 5, *tso*) is not yet invaded by bone.

In the adult (Narayanaswamy Iyer, 1943) the basioccipital is separated from the anterior 'sphenoid'.

The ossifications in the otic capsule and the tectum are clearly seen. The demarcations between the anterior prootic, dorsal supraoccipital (plus epiotic) and posterior exoccipital (plus opisthotic) are wide unossified areas in the capsule. However, in the adult these separating areas disappear.

The supraoccipital (Text-figs. 10 A, 10 B, *so*) arises perichondrally on the upper and lower aspects of the tectum connecting the posterior portion of the otic capsules. The anterior part of the processus anterior tecti (*pat*) is left unossified and between the parietals and the supraoccipital, on each side of the process, there is a gap where the cranial cavity is covered over by connective tissue. The epiotic (*epo*), with which the supraoccipital is continuous, is noticed in the sinus superior region (the posterior part of the anterior semicircular canal (Text-fig. 10 A, *asc*) and the superior part of the posterior semicircular canal (*psc*)) and extends medially as far as the inferior opening of the posterior semicircular canal, enclosing the endolymphatic foramen (*ef*).

The exoccipitals (Text-figs. 10 A, 10 B, *exo*) ossify perichondrally in the two occipital arches and each exoccipital unites with the opisthotic of its side to form a composite bone—the oto-occipital. The extension of each opisthotic is as follows: posterior face of cochlear portion (Text-fig. 10 B, *op*), behind fenestra vestibuli and below the crista parotica and in the posterior part of lateral (Text-fig. 16 A, *op'*) and inferior portion of posterior semicircular canal (Text-fig. 16 A; Text-figs. 10 A, 10 B, *op''*). Dorsally to the anterior hypoglossal foramen the opisthotic (Text-fig. 16 B, *ope*, *exo*; Text-fig. 10 B, *op*, *op''*, *exo*)

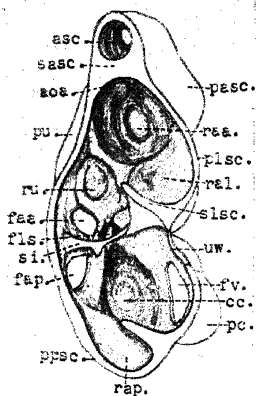


FIG. 15 A.

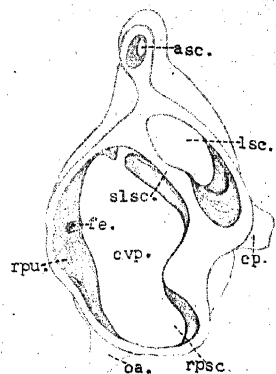


FIG. 15 B.

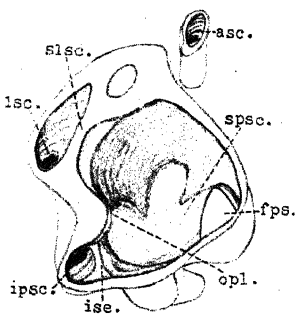


FIG. 15 C.

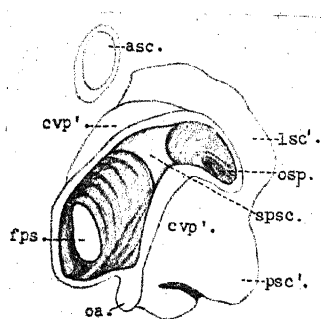
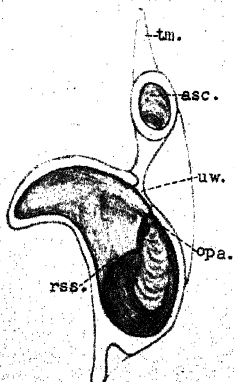


FIG. 15 D.



unites with the exoccipital. The foramen rotundum (Text-fig. 16 B, *fro*) is bounded, when viewed in the ventral aspect of the skull, anteriorly by opisthotic, posteriorly by exoccipital (*exo*), laterally by both these, and medially there is the cartilaginous basal plate. The opisthotic (Text-fig. 10 A, *op*"') when viewed on the medial aspect of the skull, forms the posterior boundary of the foramen rotundum (*fro*) and making an arch reaches the dorsal part of the exoccipital (*exo*).

TEXT-FIGS. 15 A-15 F.

15 A. Anterior part of the otic capsule of 8.0-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.), model viewed from the posterior aspect,  $\times 50$ . 15 B. Slightly posterior to fig. 15 A, viewed from the posterior aspect,  $\times 50$ . 15 C. Posterior to fig. 15 B, viewed from the anterior aspect,  $\times 50$ . 15 D. Same as fig. 15 C, viewed from the posterior aspect (only the outline position of the anterior semicircular canal is shown),  $\times 50$ . 15 E. Anterior aspect of the posterior part of the otic capsule,  $\times 50$ . 15 F. The occipito-atlantic region of a 3.6-mm. (H.-L.) embryo of *Calotes versicolor* (Daud.),  $\times 70$ . *aoa*, anterior opening of the anterior semicircular canal; *asc*, anterior semicircular canal; *bp*, basal plate; *cc*, cavum cochlearis; *cp*, crista parotica; *cvp*, cavum vestibulare posterior; *cvp'*, external aspect of cavum vestibulare posterior; *hf*, hypoglossal foramen; *faa*, foramen acusticum anterius; *fap*, foramen acusticum posterius; *fe*, foramen endolymphaticus; *fls*, lateral foramen in the intervestibular septum; *fps*, foramen pro sinu; *fv*, fenestra vestibuli; *ipsc*, inferior opening of the posterior semicircular canal; *ise*, incomplete septum; *lsc*, lateral semicircular canal; *lsc'*, external aspect of lateral semicircular canal; *oa*, occipital arch; *opa*, posterior orifice of the anterior semicircular canal; *opl*, posterior orifice of the lateral semicircular canal; *osp*, superior orifice of the posterior semicircular canal; *pasc*, prominentia ampullaris of the anterior semicircular canal; *pat*, pleurocentrum of atlas vertebra; *pac*, pleurocentrum of axis vertebra; *pc*, prominentia cochlearis; *plsc*, prominentia ampullaris of the lateral semicircular canal; *ppsc*, prominentia ampullaris of the posterior semicircular canal; *psc'*, external aspect of the posterior semicircular canal; *pu*, prominentia utricularis; *raa*, recessus ampullaris of the anterior semicircular canal; *ral*, recessus ampullaris of the lateral semicircular canal; *rap*, recessus ampullaris of the posterior semicircular canal; *rpsc*, recessus of the posterior semicircular canal; *rpu*, recessus of the utriculus; *rss*, recessus sinus superior; *ru*, recessus utriculus (in the cavum vestibulare anterior); *sasc*, septum of the anterior semicircular canal; *si*, septum intervestibulare; *slsc*, septum of the lateral semicircular canal; *spsc*, septum of the posterior semicircular canal; *tm*, posterior part of taenia marginalis; *uw*, unchondrified wall in the otic capsule.

The opisthotic is demarcated from the anterior prootic by an unossified part of the lateral semicircular canal above the fenestra vestibuli and in front of crista parotica.

Between the arch of the opisthotic (Text-fig. 10 A, *op''*), the epiotic above and the prootic anteriorly, there is an unossified area (*uo*) in the otic capsule. The prootic is an ossification in the anterior part of the otic capsule. The extension of the prootic is as follows: anterior semicircular canal as far as the middle of the gap (*gapc*) between it and otic capsule (Text-fig. 10 A, *pr''*); lateral ampullary region, anterior border of fenestra vestibuli enclosing the facial foramen (*ff*) and extending towards the basipterygoid process in the basal plate; medially it forms the posterior boundary of the foramen acusticum posterius (*fa*) (but separated from the opisthotic by an unossified portion (*uo*)).

In *Lygosoma* (Pearson, 1921) the prootic is described as extending as far as the crista parotica.

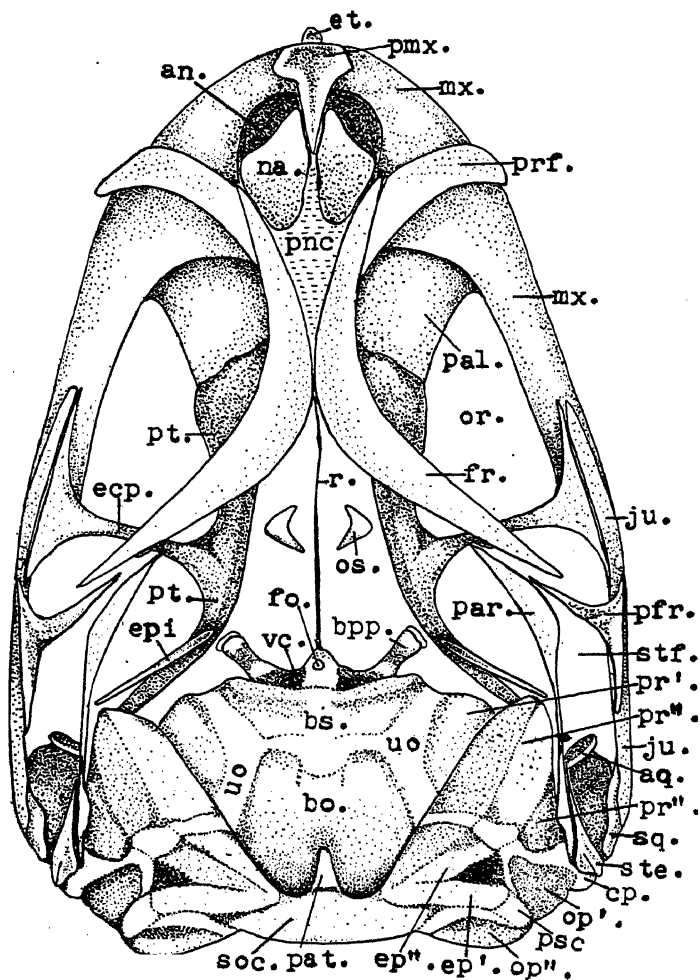
The basisphenoid in *Calotes* (Text-fig. 16 A, *bs*) appears in the crista sellaris, basitrabecular processes, and in the trabeculae near the crista; the ossification, as already noted (cf. parasphenoid), is dorsal to the parasphenoid and between this membrane bone and the basisphenoid laterally there is the parabasal or Vidian canal (Text-fig. 16 A, *vc*) through which the palatinus facialis nerve and the internal carotid artery pass. In the adult the basisphenoid fuses with the parasphenoid to form a 'sphenoid', as already described.

In *Lacerta* (de Beer, 1937) the presphenoid, basisphenoid, exoccipitals, supra-occipital, the epi-, pro-, and opisthotics and parasphenoid fuse to form a composite bone—the os basilare commune. In *Calotes* the basisphenoid, parasphenoid, ex- and supra-occipitals, epi-, pro-, and opisthotics and pleurosphenoids fuse to form a composite structure.

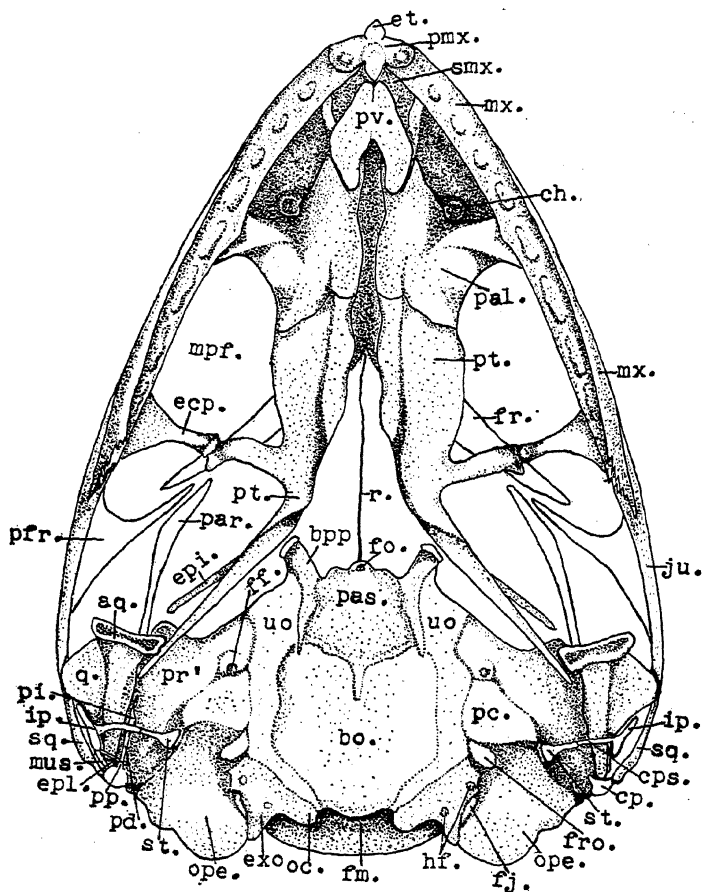
In *Calotes* the two orbitosphenoids (Text-fig. 16 A, *os*) are ossifications in the dorsal aspect of the taenia medialis.

A presphenoid is described in *Lacerta* (Gaupp, 1906) as an ossification in the posterior part of trabecula communis. I am not able to see this in sections of 8.0-mm. young of *Calotes* studied.

The pleurosphenoid ossifies in the pila antotica.



TEXT-FIG. 16A.



TEXT-FIG. 16 B.





The epipterygoid (Text-figs. 16 A, 16 B, 16 C, *epi*) or columella cranii arises as perichondral ossification in the processus ascendens. In the adult *Calotes*, as in the young stage studied, the dorsal end of the epipterygoid is connected by a ligament with the ventral aspect of the parietal right in front of the supratemporal, but Narayanaswamy Iyer (1943) has described a cartilaginous connexion as in *Varanus* (Bahl, 1937). Ventrally the club-shaped end sits in a fossa in the pterygoid. The trigeminal ganglion is slightly posterior to the epipterygoid in the cavum epiptericum (an extracranial space), and the ophthalmicus profundus V runs medially and the other two branches of the trigeminal nerve externally to the epipterygoid.

The quadrate (Text-figs. 16 A, 16 B, 16 C, *q*) bone arises as perichondral ossification in the quadrate cartilage and is free, and, therefore, streptostylic.

The articular (see Text-fig. 5, *art*) is noticed as a circular ossification round Meckel's cartilage in the processus retro-articular region. This bone undergoes fusion with an anterior membrane bone, the prearticular. In *Calotes* the prearticular is present as a separate bone only in early stages.

No mento-meckelian ossification at the symphysis of the rami of lower jaw is noticed.

The ossification in the first ceratobranchial is the cornu branchiale primum.

#### DISCUSSION.

In the posterior region of *Calotes* the basal plate shows two condyles with a notch between them and the notochord runs dorsally to the basal plate as in *Lacerta* (Gaupp, 1900). In early stages of *Eumeces* (Rice, 1920) and *Sphenodon* (Schauinsland, 1900) the notochord may be embedded posteriorly in cartilage; in *Chelone* (Gaupp, 1900) it enters the basal plate ventrally. In *Emys* (Kunkel, 1912) and *Crocodylus* (Shiino, 1914) the notochord is completely embedded.

In *Lacerta* (Gaupp, 1900) the intercondylar notch is not prominent, while in *Eumeces* (Rice, 1920) it is pronounced, and Rice homologized these condyles with the mammalian ones. In the adult *Calotes* the monocondyle includes the

proatlas, and the condylar region shows clearly this addition. Wedged in between the exoccipital (plus opisthotic) projections, the condyle is demarcated by a thin groove on each side.

This median condyle is the hypocentrum of the proatlas vertebra, while the occipital arches represent its neural arches. The pleurocentrum of this proatlas unites with the same of the atlas and axis vertebrae to form the odontoid peg. That this happens is seen in the 3.6-mm. stage of *Calotes* where the occipital region of the basal plate is in temporary confluence with its own pleurocentrum (as happens normally in the vertebral region) which is already fused with the pleurocentra of atlas and axis vertebrae, the notochord projecting centrally from this. Later, a joint appears between the hypocentral condyle fused on to the basal plate between the exoccipital projections and the atlas vertebra. Thus the craniovertebral joint is intra-vertebral and intersegmental as in other *Lacertilia* (de Beer, 1937).

In *Eumeces* (Rice, 1920) a temporary union of the odontoid tip (pleurocentrum) with the basal plate was recorded. While in *Lacertilia*, *Sphenodon*, and *Ophidia* the condyle is hypocentral, the pleurocentrum forms the median condyle in *Crocodylia* and *Chelonina*.

The fissura metotica is undivided in *Lacerta* (Gaupp, 1900), while in *Eumeces* (Rice, 1920) the fissure is cut into two by the close apposition of the posterior ampullary prominence and the basal plate. The more anterior is the recessus scalae tympani lateralis, while the posterior one is the foramen jugulare for the exit of X and XI cranial nerves. Through the former the glossopharyngeal nerve comes out. In *Calotes* also the fissura is separated into two by the coming together of the basal plate with the prominentia ampullaris posterior with only a thin connective tissue lamella separating them, in the region of the hypoglossal foramina. The posterior jugular foramen is itself divided into an anterior larger and a posterior smaller part which transmits nothing. In the adult a part of the recessus scalae tympani lateralis becomes the fenestra rotunda, over which the secondary tympanic membrane is stretched, and

through this the glossopharyngeal nerve gets out. This arrangement is as in other lizards.

The number of hypoglossal foramina varies; in *Calotes* there are two as in *Eumeces*, *Platydictylus* and *Sphenodon* (four in early stages), and later stages of *Chrysemys*; in *Lacerta*, *Crocodylus*, and early stages of *Chrysemys* there are three, and four in *Tropidonotus*. Rice (1920) has given a list of the number of hypoglossal foramina in various reptiles.

The septum intervestibulare divides the internal cavity of the otic capsule into an anterior smaller (cavum vestibulare anterius) and a posterior larger chamber (cavum vestibulare posterius) in lizards. In *Calotes* the median orifice is not limited as in *Lacerta*. The utriculus establishes connexion with its anterior portion by passing over the septum intervestibulare towards its dorsomedian aspect, i.e. internally to the septum semicirculare laterale (Text-fig. 15 A). This is unusual. The lateral orifice is normally situated.

In the orbitotemporal region of *Calotes* the formation of the interorbital septum is interesting. In the earliest stage studied (3.6 mm.) it is noticed that the preoptic roots are separate and the orbital cartilages connect each preoptic root with the pila antotica of its side (Text-fig. 1); the pila metoptica unites with the orbital cartilage in its region so that a typical fenestra metoptica is formed. In the next stage (6.0 mm.) a short interorbital septum (Text-fig. 2) has appeared which anteriorly shows forking. From the lower ends of this fork the two sphenethmoid commissures start, while from the upper the two preoptic roots proceed and meet the orbital cartilages. The two pilae metopticae connect the orbital cartilages posteriorly and there is no antotic connexion. In the 7.0-mm. stage the forked nature of the interorbital septum is lost, and, therefore, the sphenethmoid commissures diverge from the ventral end of the interorbital septum (Text-fig. 3); the two preoptic roots have united to form the planum suprasedale. An additional pillar reaching from the ventral portion of the interorbital septum to the posterior part of the planum separates an anterior septal fenestra from a posterior optic. The paired

nature of the metoptic pila is lost; the taeniae mediales arising from the posteroventral border of the planum meet the united cartilago hypochiasmatica, subiculum infundibuli and pilae metopticae. In the next stage (8.0 mm.) the preoptic pillar is elongated and the ventral unpaired portion of the interorbital septum has increased in size.

In *Lacerta* (de Beer, 1930) the orbital cartilages do not unite with paired preoptic pillars and even in the earliest stage, (4.5 mm.) showing the planum supraseptale (which represents the anterior portion of orbital cartilages), it is single; only posteriorly by means of the taenia medialis, which represents the orbital cartilages in this region, the trabecula is connected with the paired pila metoptica. In *Calotes*, on the other hand, the preoptic roots are separate and these are connected with the pila antotica by the orbital cartilages to start with, and later, when the interorbital septum appears and the eyes enlarge, the preoptic roots fuse to form a pillar, the two orbital cartilages unite to form the planum supraseptale, and the posterior portion of the orbital cartilages (taenia medialis) fuse with the median pillar (united cartilago hypochiasmatica, subiculum infundibuli and pilae metopticae). The antotic connexion is lost and the taeniae marginales are also absent.

In *Anguis* (Zimmermann, 1913) the planum supraseptale is composed of paired separate orbital cartilages.

The fenestra metoptica in *Calotes* is incomplete in the 8.0-mm. stage, there being no connexion between the pila metoptica and pila antotica by a taenia medialis as in *Lacerta* (Gaupp, 1900). In *Eumeces* (Rice, 1920) also the fenestra metoptica is incomplete, the pila antotica being barely indicated.

The pila antotica ossifies, and it has been called the 'alar process' by previous workers (Siebenrock, 1892, 1894; Bahl, 1937). This is clearly the pleurosphenoid, which also becomes united with the various bones in this region of *Lacerta* to form an 'Os basilare commune'. In *Calotes* also a pleuro-sphenoid is noticed, but the composite sphenoid bone is separate from the basioccipital.

The ethmoid region of *Calotes* stands apart from other lizards described. The lamina transversalis anterior is uncon-

nected on its lateral aspect with the roofing parietotectal cartilage, so that a zona annularis, as seen in *Lacerta*, is absent in *Calotes*.

The nasolachrymal duct, however, opens into the choanal region behind the lamina transversalis anterior, which is also the condition in *Lacerta* (Gaupp, 1900).

The lamina orbitonasalis unites with the anterior portion of paranasal cartilage in *Calotes*, and a concha nasalis, so characteristically seen in lizards, is absent in *Calotes*. In this it resembles *Sphenodon*. Further, no lateral fenestra is seen in *Calotes*, and in the side view the nasal septum and ectochoanal cartilages are seen (Text-fig. 14 c). The lateral nasal glands are accommodated in a space—the cavum conchale, between the posterior portions of the paranasal and parietotectal cartilages; in other lizards the glands are enclosed in a cartilaginous concha nasalis.

Starting from the median aspect of the lamina transversalis anterior and meeting the median wall of the lamina orbitonasalis is the paranasal cartilage in *Lacerta*, which is unconnected with the nasal septum. In *Calotes* this cartilage is wanting; there is only a short projection from the median aspect of lamina orbitonasalis.

The lamina orbitonasalis is demarcated from the nasal capsule by the epiphaneal foramen and the processus maxillaris anterior is seen anterolaterally from the lamina. While a posterior maxillary process is not visible in the 8.0-mm. stage embryo, in the young of *Calotes* of the same head-length two cartilaginous parallel projections run dorsally to the palatine from the lamina orbitonasalis. The more ventral of these is the processus maxillaris posterior while the other is without significance. In *Lacerta* (Gaupp, 1906), also, on the dorsal aspect of the palatine, isolated nodules of cartilage in line with the processus maxillaris posterior are recorded.

In *Calotes*, on the posterior aspect of the lamina orbitonasalis, on each side of the interorbital septum, there is a small cartilaginous projection, and I have called it the posterior laminal process.

The pterygoquadrate (Text-fig. 11) is represented as a

separate movable quadrate posteriorly, a processus ascendens (which ossifies into the epipterygoid) and a basal process (the meniscus pterygoideus; the metapterygoid of fishes) articulating with the basitrabecular process; no processus pterygoideus is noticed. However, in earlier stages, from the region where later the processus ascendens appears, there is an anteriorly directed mesenchymatous strand representing the processus pterygoideus. This does not chondrify. In *Lacerta* there is a processus pterygoideus united with the processus ascendens, which is always separate from the quadrate, and in *Eumeces* (Rice, 1920) the processus pterygoideus is independent of the processus ascendens; in earlier stages of *Eumeces* the processus ascendens and processus pterygoideus and quadrate are united. In *Mabuia*, *Zonurus*, and *Eremias* (Broom, 1903) there is a persistent cartilaginous connexion between quadrate and epipterygoid. In *Sphenodon* (Howes and Swinnerton, 1901), *Chrysemys* (Shaner, 1926), and crocodile (Parker, 1883) these processes are united with the quadrate cartilage. According to Fürbringer (1904), to ensure greater mobility of the jaw, the quadrate becomes separated off from its anterior connexions. Characteristically the quadrate of the snake is also free from all these processes.

The columella auris shows, in the young *Calotes* with the head-length of 8.0 mm., an ossified stapes and a cartilaginous extracolumella. From the insertion plate which fits into the tympanic membrane there arise the characteristic processes: the pars inferior and pars superior. From the latter arise the processus accessorius anterior and posterior. Arising from the pars superior there is a ligament passing into the processus paroticus and a muscle which is a part of the stylomastoid muscle inserted on the crista parotica.

From the processus accessorius anterior there is a ligament attaching itself to the quadrate; such a structure is also described in some chelonians by Fuchs. From the connecting cartilaginous piece between the insertion plate and stapes in *Calotes* there arises the processus internus which articulates with the quadrate and a ligamentary processus dorsalis which meets the processus paroticus.

The processus accessorius anterior connexion with the quadrate is not noticed in *Lacerta* (Gaupp, 1900) and *Eumeces* (Rice, 1920).

Generally the processus accessorius posterior or processus interhyalis unites with the ceratohyal in early stages and this connexion may even persist in later stages. In early stages of *Lacerta* (Hoffmann, 1889; Gaupp, 1900), *Eumeces* (Rice, 1920), *Tropidonotus* (Rathke, 1830), and *Crocodylus* (Parker, 1883) the connexion is noticed. In *Sphenodon* (Howes and Swinnerton, 1901) the connexion is permanent. There may be a mesenchymatous connexion between the pars interhyalis and ceratohyal in *Chelonia* (Bender, 1911; Smith, 1914); in *Emys* (Kunkel, 1912), however, there is a ligament running between the pars interhyalis and the retroarticular process of the lower jaw.

A processus dorsalis is noticed in *Lacerta* (ligamentary, Gaupp, 1900) and *Calotes*. In *Eumeces* (Rice, 1920) and *Lygodactylus* (Brock, 1932) it is absent. In *Agama* (Brock, 1932) it persists as a cartilaginous connexion. In *Varanus*, topographically, a ligamentary processus dorsalis is present, though Bahl (1937) noted to the contrary. In *Sphenodon* (Howes and Swinnerton, 1901; Versluys, 1903) the dorsal process is united with the quadrate; in *Tropidonotus* an independent chondrification acquiring connexion with the quadrate is homologized with the processus dorsalis (de Beer, 1937). While it is absent in *Chelonia* (Kunkel, 1912), in *Crocodylus* it fuses with the quadrate.

A cartilaginous processus internus projects towards the quadrate in *Calotes*, and as in *Lacerta* (Gaupp, 1900) there is no union with the quadrate (see, however, de Beer, 1937, p. 225). In *Eumeces* (Rice, 1920) it is very short and in *Lygodactylus* (Brock, 1932) it is absent. So also in *Sphenodon* (Howes and Swinnerton, 1901), *Tropidonotus* (Parker, 1879), *Emys* (Kunkel, 1912), and *Crocodylus* (Goldby, 1925).

The last ligamentary connexion is the one that starts from the pars superior of the insertion plate and gets inserted on the processus paroticus at the region where the processus dorsalis

ligament also meets it in *Calotes*. The chorda tympani runs dorsolaterally to this ligament, while the stapelial artery runs medially to the processus dorsalis. In describing this tendon, Rice (1920) recorded its presence in *Eumeces* (with the chorda tympani running dorsally to it) and noted: 'This "tendon of extracolumella" is carefully described by Versluys (1898) for *Sphenodon* and adult lizards; it was only lacking in *Amphisbaena* among the many lizards studied.' In *Varanus* Bahl (1937) described it running into the crista parotica, but the chorda tympani had no relation either with the processus dorsalis ligament or this. Probably on account of this, he denied the presence of a processus dorsalis. In *Agama* (Brock, 1932) there is the extracolumella-crista ligament, but the chorda tympani does not loop round it. All the same Brock noted: 'From the condition in *Lygodactylus* where there is a cartilaginous connexion between the extracolumella and the crista parotica in early stages it may be judged that the ligament of *Agama*, *Lacerta*, etc., is homologous with the cartilaginous and ligamentous connexion of the Geckos and the latter would then be the more primitive condition.' In all the examples (see below) where such a ligamentary or cartilaginous (laterohyal) connexion is noticed, the chorda tympani always runs dorsolaterally to it; while the ligamentous connexion of *Calotes* and *Eumeces* may be homologous with the laterohyal, that of *Agama* and *Varanus* must be considered analogous.

In *Sphenodon*, from the processus paroticus or intercalary there is a laterohyal cartilage uniting with the extracolumella enclosing a 'Huxley's' foramen between the processus dorsalis and the laterohyal; the chorda tympani runs laterally to it. There is in addition to this a ligament from the extracolumella to the crista parotica and the exact homology of this tendon when there is a permanent laterohyal connexion with the ceratohyal becomes difficult, and Versluys assumed that the connexion of the hyoid arch has been secondarily regained. A similar laterohyal connects in *Crocodylus* (Versluys, 1903) the intercalary with the epihyal, which itself is in contact with the extracolumella by means of the pars interhyalis; the chorda



tympani runs laterally to the laterohyal (de Beer, 1937, Pl. 141, fig. 17).

In Geckos with the ligamentary connexion between the extracolumella and parotic process (which may be cartilaginous in early stages, e.g. *Lygodactylus* (Brock, 1932, fig. 6 B)) the ceratohyal is directly connected with the parotic process, with the chorda tympani bearing no relation to it (or by-passing laterally to it, de Beer, 1937, Pl. 141, fig. 13), apparently resembling a laterohyal. However, in early stages the extracolumella is connected both with the processus paroticus and ceratohyal.

A brief reference may be made to the muscle that arises from the pars superior and gets inserted on the crista parotica. Externally to the ligament referred to above, the muscle has been noticed in *Lygodactylus* also (Brock, 1932). It is noted by the author that the muscle is a part of the stylomastoid and not depressor mandibulae as Versluys would have it. Having examined early and late stages of *Calotes*, I am in agreement with Brock.

#### SUMMARY

1. In the earliest stage of *Calotes* studied, the basal plate is confluent with the pleurocentrum of the atlas and axis vertebrae. Later, a joint appears between the hypocentral condyle and the first vertebra. This shows that, at least temporarily, the elements of the anterior sclerotomic half in this region are in continuity with the posterior in front as happens in the vertebral region. The occipito-atlantic joint is, therefore, intra-vertebral and intersegmental as in other *Lacertilia*.

2. The anterior semicircular canal is completely separated for a short distance from the remaining otic capsule. The gap is filled with connective tissue.

3. The intervestibular septum shows a lateral foramen which transmits nothing and the utricular connexion between the anterior and posterior chambers passes posteriorly to the median part of the septum and, therefore, a medial orifice is not formed.

4. The preoptic roots, the orbital cartilages, and metoptic pila are paired in early stages; the orbital cartilage connects the

preoptic root, pila metoptica and pila antotica dorsally. Later the two preoptic roots merge to form a median preoptic pillar, the orbital cartilages anteriorly unite to form the planum suprasedale, while posteriorly also the orbital cartilages (taenia medialis) unite at the region of the hypophysial foramen. This posterior united portion is met by a median vertical pillar (formed by the fusion of cartilago hypochiasmatica, subiculum infundibuli, and pilae metopticae) arising from the trabecula communis.

The single septal fenestra is divided into an anterior larger and a posterior optic by the formation of median interorbital pillar from the ventral interorbital septum which meets the posterior portion of the planum suprasedale. The ventral portion of the interorbital septum is never noticed to be paired; the taenia marginalis is absent. However, short projections from the posterodorsal margin of the planum and from the anterodorsal face of the otic capsule represent the reminiscence of marginalis connexion. A supratrabecular bar is absent.

5. In the nasal capsule, a concha nasalis is absent; therefore, the lateral nasal glands are unenclosed in a cartilaginous capsule. The anterior portion of the paranasal cartilage unites with the dorsal portion of the lamina transversalis anterior, and the latter gives rise to an ectochoanal cartilage, but a paraseptal cartilage is absent. On the ventral side, from the free median margin of the lamina orbitonasalis, there arises a short projection which represents the posterior portion of the paraseptal cartilage.

6. The pterygoquadrate shows a free streptostylic quadrate, a processus ascendens which ossifies into the epipterygoid, a processus pterygoideus only in early stages, a basipterygoid articulation by a free meniscus cartilage, and an otic articulation with the crista parotica and processus paroticus by the quadrate.

7. The columella auris shows a ligamentary processus dorsalis connexion with the processus paroticus, a cartilaginous processus internus which articulates with the quadrate, a processus accessorius anterior which is connected with the quadrate by a ligament, and a ligamentary connexion between the pars superior of the insertion plate and processus paroticus. The

processus accessorius posterior-ceratohyal connexion was not noticed. There is also a muscle (a part of M. stylohyoid) spanning the pars superior and crista parotica. The pars superior-paroticus ligamentary connexion, with the chorda tympani running laterally to it, is homologized with the laterohyal of *Sphenodon* and the crocodile.

8. The hyoid apparatus shows a processus lingualis and cornuhyale (paired hypo- and ceratohyals) arising from a median basihyal and two pairs of ceratobranchials.

9. In the osteocranium, the oto-occipital of each side is formed by the fusion of opisthotic and exoccipital, while the supraoccipital is formed by an ossification in the tectum and its fusion with the two epiotics formed in the sinus region of the otic capsule. The basioccipital and the composite 'sphenoid' are not united. The pleurosphenoid ossifies in the pila antotica. The epipterygoid is connected at its dorsal end with the parietal by a ligament, and ventromedially it is free from the meniscus cartilage. The frontals and parietals are paired in the stage examined, and in the adult the parietals of each side fuse, as also the frontals.

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# The Development of the Olfactory Organ of *Kaloula borealis* (Barbour) as compared with that of *Rana nigromaculata* Hallowell

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With 7 Text-figures

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In a previous paper on the development of the olfactory organ in *Rana nigromaculata* by one of the present authors (Tsui, 1946a) questions were raised about the significance of the ephemeral existence of the lateral appendix and about the functions of different kinds of nasal glands and of Jacobson's organ or the recessus medialis. To elucidate them experimental studies will be necessary. Unfortunately the species *nigromaculata* which is abundant in North China is very rare in this part of the country. *Kaloula borealis*, on the other hand, is very common here. In order to use it as experimental material, familiarity with the developmental history of its nasal organ is prerequisite. In addition, it is of interest to compare the development of this organ in *Rana nigromaculata* and *Kaloula borealis*, and to see whether certain findings in the former species concerning certain controversial points will be confirmed in the latter.

The results of a complete study of the development of the nasal organ in *Kaloula borealis* and of its comparison with that of *Rana nigromaculata* are embodied in the present paper.

For the sake of shortness the two species will be generally referred to by their generic names only.

## MATERIAL AND TECHNIQUE

*Kaloula borealis* is very abundant in Kunming. The animals stay in their subterranean burrows in the dry months



of the year but come out to breed in the rainy season (June to August). Their eggs are found on the surface of shallow and temporary pools but are very rare in the deeper ponds or paddy fields where eggs of toads and other frogs are commonly found. They form loose masses and are easily separated from one another by a water current. They are easily distinguishable from other amphibian eggs by the cap-shaped jelly layer at the animal pole. From eggs hatched in the laboratory a series of young larvae, 2-10 mm. in body length, was preserved. Larvae of later stages were collected from the field and immediately preserved. They are identifiable by the absence of the external naris, papillae, horny beak, and horny teeth around the mouth. The larger larvae (24 mm. long or over) are further characterized by possessing a fine whitish line running from the nose to the eye on each side of the head (Boring, Liu and Chou, 1932). This characteristic feature makes the identification easier, as it can be seen with the naked eye. For fixation, Spuler's fluid gave most satisfactory results. Bouin's fluid caused some shrinkage and was not used after a preliminary trial. Serial cross sections were cut  $8\mu$  in thickness and stained with Delafield's hematoxylin and eosin. For the purpose of differentiating the ectoderm and endoderm in the primitive oral cavity, serial sagittal sections were cut  $5\mu$  in thickness, and stained with Heidenhain's hematoxylin.

#### DEVELOPMENTAL ANATOMY

##### A. Olfactory Cavities.

The development of the olfactory organ in *Kaloula borealis* is similar in general outline to that of *Rana nigromaculata*. It is, however, comparatively simple and may be conveniently divided into three stages of four as in the case of *Rana*.

1. First Stage.—From the inception of the olfactory anlage to the formation of the primitive choana (length of larva, 3-7.5 mm.). The method of formation of the olfactory anlage is similar to that in *Rana*, only it appears a little later in life (length of larva, 3mm.). Following this the olfactory pit appears. In the same

manner as in *Rana* the pit extends upward and inward to form the dorsal and middle lumen. The neighbouring cells behind the dorsal lumen are then differentiated to form the lateral appendix. As in *Rana*, posterior to the lateral appendix the olfactory placode is divisible into two portions—a thin lateral one and a thick median one—between which there is a series of clefts. These merge later with one another to form a narrow cavity which becomes continuous with the middle lumen. This cavity corresponds in position to the ventral lumen in *Rana*, but the inward projection which marks off the middle lumen from the ventral lumen in *Rana* is wanting in this species. Therefore, this cavity can be regarded only as a posterior extension of the middle lumen.

The so-called oro-nasal groove which Kurepina (1931) claims to be present in the amphibian larvae was not found in *Rana*. In *Kaloula* there is also no such structure.

The mode of fusion of endoderm and ectoderm at each side of the pharyngeal membrane in the roof of the primitive oral cavity is exactly the same as found in *Rana*. The primitive choana opens into the endodermal part of the oral cavity (when the larva is 6 mm. long) in the same manner as in that species.

During the opening of the primitive choana the olfactory placode extends posteriorly to form a prolongation beyond the primitive choana. It is small and solid at first, but soon lengthens and becomes hollow containing a lumen which constitutes a further extension of the posterior part of the middle lumen.

The external naris is formed immediately after the formation of the primitive choana (length of larva, 6.5 mm.). As in *Rana* there is also a short entrance canal which connects the olfactory organ with the external naris. This opening exists, however, only for a short while in *Kaloula* and closes up when the larva is only 7.5 mm. long. During the greater part of larval life there is no external opening to the nasal organ. The communication of the nasal organ with the outside is through the mouth only. As will be described later, external nares are formed afresh at the beginning of metamorphosis.

2. Second Stage.—Opening up of the middle lumen and formation of the blind sacs (length

of larva, 7.5-48 mm.). The olfactory placode enlarges and its floor becomes longitudinally split in the middle. This separation begins from the end next to the primitive choana and extends anteriorly to the front-most part of the placode (Text-figs. 1 A and 2). As a result, the middle lumen now opens up along its entire ventral side into the oral cavity and forms together with the primitive choana a simple opening at this stage. This large and compound opening is designated as the choana, since through it the olfactory organ communicates with the oral cavity. The posterior prolongation described in a preceding paragraph remains as a blind sac behind the choana (Text-fig. 1 B).

In *Rana* the primitive choana is the terminal opening of the ventral lumen. This position remains unchanged throughout the whole of its embryonic life. The position of the primitive choana in *Kaloula* corresponds to that in *Rana* at an early stage. However, as soon as the posterior prolongation lengthens out from the olfactory placode and becomes a blind sac, its position is no longer terminal. Then, too, the opening of the middle lumen into the oral cavity obscures its identity, making the compound opening (the opened middle lumen plus the primitive choana) look like an enlarged primitive choana. Thus, the existence of a sac posterior to it and its confluence with the middle lumen tend to make the primitive choana appear shifted forward and extended anteriorly, but in reality it remains in its original position as in the case of *Rana*.

To our knowledge, the opening of the middle lumen direct into the oral cavity is found only in this species. The significance of it is unknown, but it is interesting to note that this change which enables water in the oral cavity to come in easy contact with the olfactory placode is synchronous with the closing up of the external naris.

There are also three embryonic blind sacs at the end of this stage—the anterior lower sac, upper sac, and posterior lower sac.

The anterior lower sac arises from the ventro-anterior region of the olfactory placode (length of larva, 10 mm.; Text-fig. 2) as found in *Rana*. The upper sac, and the posterior lower sac arise in the following manner.

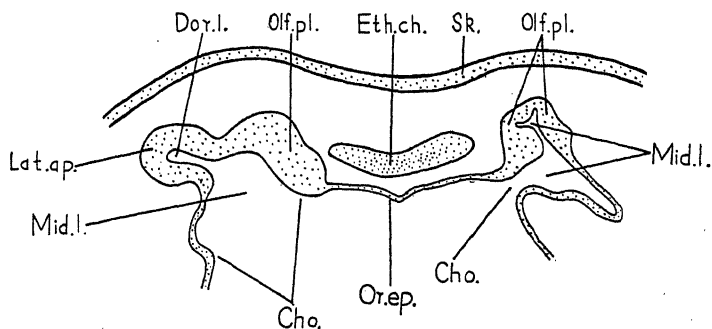


FIG. 1 A

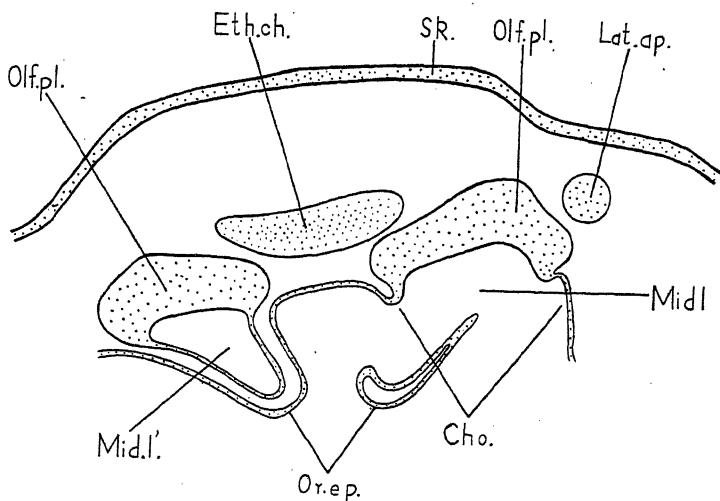
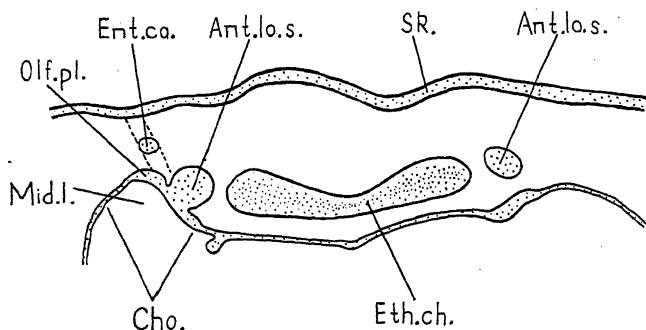


FIG. 1 B

## TEXT-FIG. 1 A and B.

- A. Cross section through the olfactory organ, showing the opening of the middle lumen into the oral cavity (length of larva, 8 mm.). This section is slightly oblique; the left olfactory organ is cut through the most anterior region and the right one through the middle region. *Cho*, choana; *Dorl*, dorsal lumen; *Ethch*, ethmoidal part of chondrocranium; *Latap*, lateral appendix; *Midl*, middle lumen; *Olfpl*, olfactory placode; *Orep*, oral epithelium; *Sk*, skin.  $\times 120$ .
- B. Cross section through the same specimen as A but posterior to it. *Midl*, posterior extension of the middle lumen; *Olfpl*, posterior prolongation of the olfactory placode. Other lettering as in A.  $\times 120$ .

At the end of the first stage when the naris is closed up the lumen of the distal part of the entrance canal is obliterated, but its wall remains intact persisting as a fine cord (Text-fig. 2). The proximal part of the canal, which is connected with the olfactory placode, remains open, though the lumen is very small. When the larva reaches the length of 11 mm. this part together with the adjacent part of the placode enlarges to form a small dorsal



TEXT-FIG. 2.

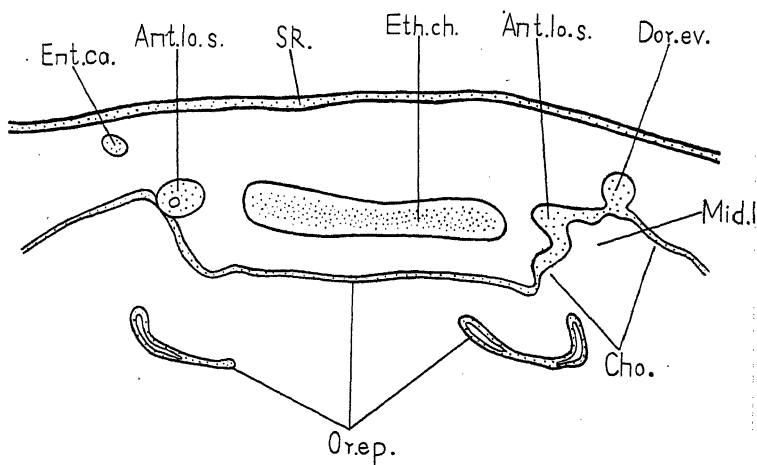
Cross section through the anterior region of the olfactory organ, showing the anterior lower sac (*Antilos*) and the closed entrance canal (*Entca*) on the right side; the dotted lines indicate its connexion with the closed external naris anterior to this section and with the olfactory placode posterior to the same (length of larva, 10 mm.). Other lettering as in Text-fig. 1 A.  $\times 120$ .

evagination (Text-fig. 3). The closed portion of the canal persists and is connected with the dorsal evagination. As the dorsal evagination enlarges, it forces open again a portion of the closed entrance canal, so that its length decreases accordingly (Text-fig. 4 A).

Later (length of larva, 17 mm.) the posterior part of the dorsal evagination together with the upper part of the olfactory placode extends medially, and there appears a median groove between the upper and lower part of the placode (Text-fig. 4 B). This groove is at first limited to the anterior portion, but later, as the placode enlarges, it extends posteriorly and at the same time widens into a trough which will be called the median trough.

Finally (when the larva is 40 mm. long), it extends to the end of the posterior prolongation of the placode.

During the extension of the median trough the lower part of the placode expands laterally (length of larva, 27 mm.). As a result, a trough on the other side or a lateral trough is formed (Text-fig. 5 A and B). It extends also posteriorly and meets the median trough at the end of the posterior prolongation of the



TEXT-FIG. 3.

Cross section through the anterior region of the olfactory organ, showing the dorsal evagination (*Dorev*) on the left side, the section is slightly oblique, the section of olfactory organ on right side anterior to that on the left (length of larva, 13 mm.). Other lettering as in Text-figs. 1 A and 2.  $\times 120$ .

placode, thus dividing the placode into two compartments, which are henceforth known as the upper sac and the posterior lower sac with the choana as the opening of the latter (Text-fig. 5 A and B).

The formation of the upper and posterior lower sacs in the embryonic olfactory organ of *Kaloula* as described above is similar to that found in *Rana*. But in *Rana* there appears at a very early stage an inward projection which later forms a lateral groove dividing the olfactory placode early into upper

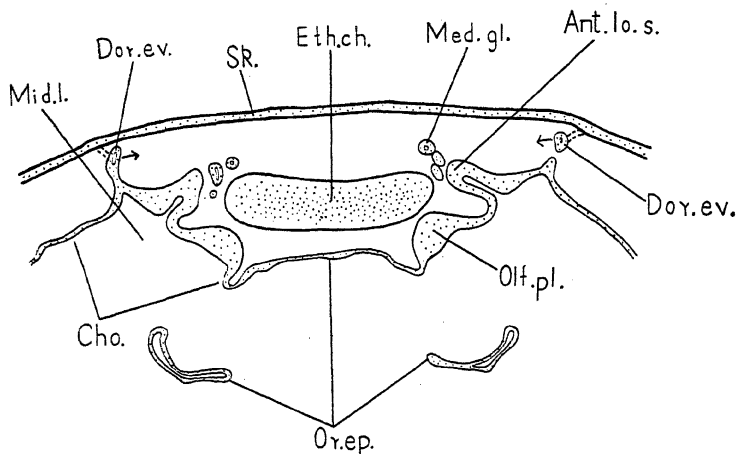


FIG. 4 A

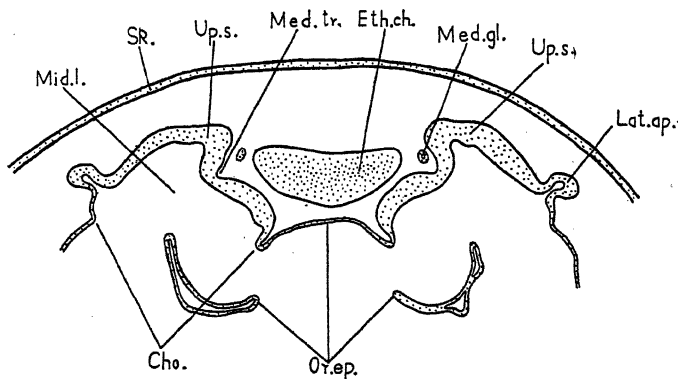


FIG. 4 B

TEXT-FIG. 4 A and B.

- A. Cross section through the anterior region of the olfactory organ, showing the enlargement of the dorsal evagination. The arrows indicate the direction of its extension immediately posterior to this section; the dotted lines show the position of the closed entrance canal anterior to this section (length of larva, 29 mm.). *Antlos*, anterior lower sac; *Dorev*, dorsal evagination; *Medgl*, median nasal gland. Other lettering as in Text-fig. 1 A.  $\times 60$ .
- B. Cross section through the middle region of the olfactory organ, showing the median trough (same specimen as A but posterior to it). *Medgl*, median nasal gland; *Medtr*, median trough; *Ups*, upper sac. Other lettering as in Text-fig. 1 A.  $\times 60$ .

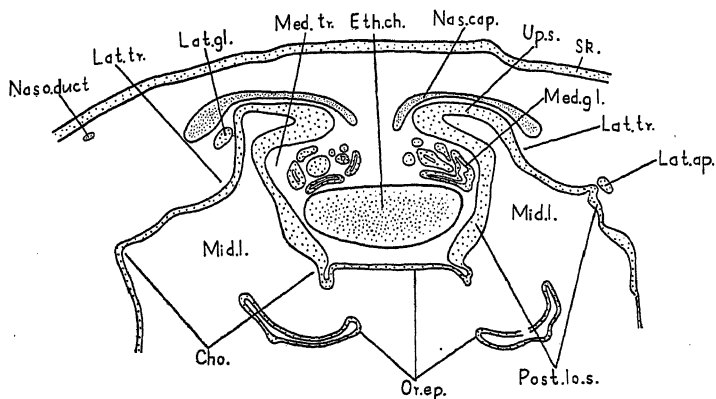


FIG. 5 A

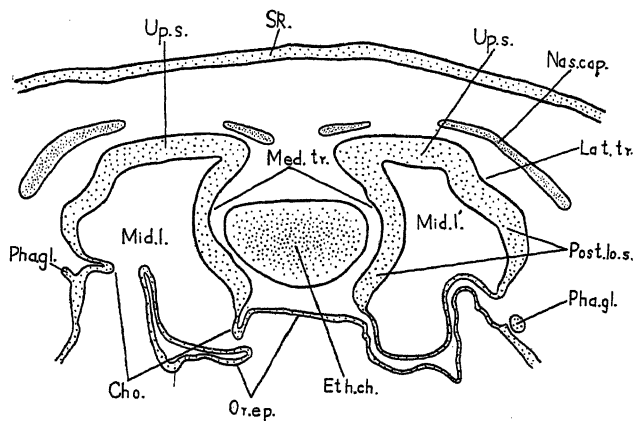


FIG. 5 B

## TEXT-FIG. 5 A and B.

- A. Cross section through the anterior region of the olfactory organ (length of larva, 42 mm.). *Latgl*, lateral nasal gland; *Lattr*, lateral trough; *Medgl*, median nasal gland; *Medtr*, median trough; *Nascap*, cartilaginous nasal capsule; *Naso duct*, naso-lachrymal duct; *Postlos*, posterior lower sac; *Ups*, upper sac. Other lettering as in Text-fig. 1 A.  $\times 40$ .
- B. Cross section through the posterior region of the olfactory organ of the same specimen as A. *Midl*, posterior extension of the middle lumen; *Phagl*, pharyngeal gland. Other lettering as in A, and in Text-fig. 1 A.  $\times 40$ .



and posterior lower sac on the outer side. In *Kaloula* no such lateral groove is found. The upper sac is not marked off from the posterior lower one until the median trough appears. The lateral trough appears still later, which corresponds to the supra-lateral groove in *Rana*. In that species when the upper sac enlarges there is also formed below it a similar medial trough. It is clearly shown in the model depicted in figs. 20-2 of the previous paper (Tsui, 1946a), but unfortunately the median view of the model which would have shown the median trough was not depicted.

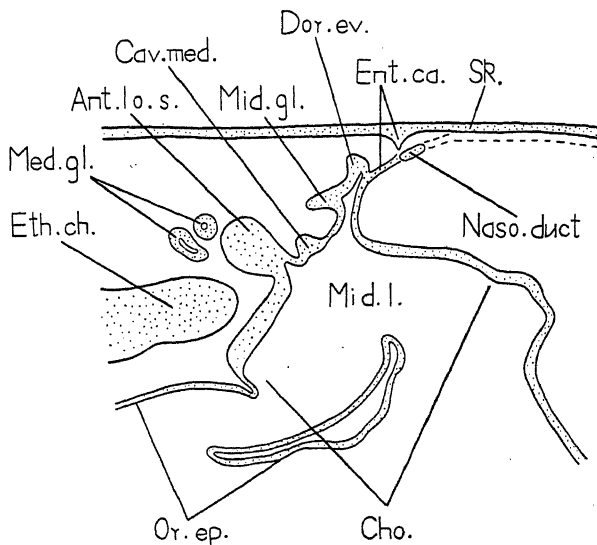
In *Rana* there exists a ledge, between the two grooves—the lateral and superlateral grooves. This ledge together with the lateral groove below it is absent in *Kaloula*. The ledge in *Rana* is the anlage of the recessus lateralis, which in the case of *Kaloula* grows out directly from the side extension of the *cavum principale* during the metamorphosis.

When the larva grows to the length of 33 mm. there arises another small evagination in the antero-median region of the upper sac (Text-fig. 6). This region corresponds to the lower part of the enlarged entrance canal in *Rana* at a point above the anterior lower sac. The evagination lengthens medially and laterally (Text-fig. 7 A and B), and becomes the *cavum medium* in the adult stage. It arises in the same manner as the same structure in *Rana*.

It may be pointed out here that the mode of formation of all the blind sacs in the embryonic olfactory organ of these two species is almost the same. Only, in *Kaloula* the closure of the external naris and of the distal part of the entrance canal and the absence of the lateral groove together with the ledge above it are responsible for minor differences.

3. Third Stage.—From the beginning of the metamorphosis to the adult stage (48 mm. larva to young frog). When the tadpole reaches 48 mm. in length, its tail begins to be absorbed. The shrinkage in size both of the body and of the olfactory organ is more pronounced than in *Rana*. There is also a similar shifting of the embryonic nasal organ to the tip of the head.

The first remarkable change is the formation of the external naris. At the end of the second stage the dorsal evagination grows actively upwards (dorsad, Text-fig. 6) until it reaches the

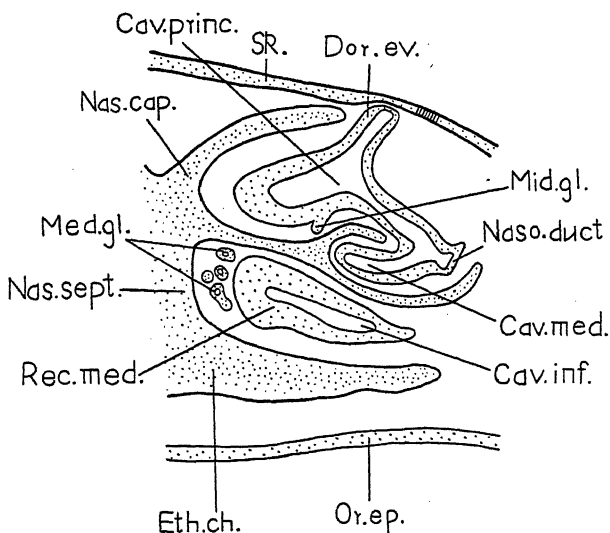


TEXT-FIG. 6.

Cross section through the anterior region of the left olfactory organ, showing that the closed entrance canal is now ruptured. Its distal end is attached to the skin at the spot where the external naris existed. Its proximal part is now connected with the naso-lachrymal duct. The section is slightly oblique, so that the distal end of the closed entrance canal, which is a little bit anterior to the proximal end, is shown in the same section; the dotted lines indicate the epidermal ridge (anlage of the naso-lachrymal duct) posterior to this section. It runs along the skin but begins to get detached at its proximal end (length of larva, 38 mm.). *Antlos*, anterior lower sac; *Cavmed*, cavum medium; *Entca*, closed entrance canal; *Midgl*, middle nasal gland. Other lettering as in Text-figs. 1 A and 5 A.  $\times 60$ .

skin at a point median to the former (now obliterated) external naris (Text-fig. 7 A). At the beginning of present stage the point of contact between the dorsal evagination and the skin is broken through; thus a new external naris is formed (Text-fig. 7 B). That the external naris is formed *de novo* and is not the old

one now reopened is clearly demonstrable. During the second stage the spot in the skin where the former external naris existed can be located by its connexion with the remnant of the



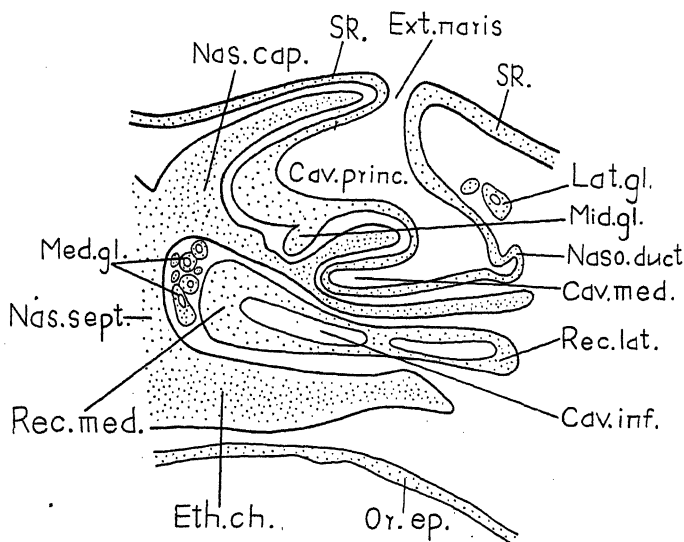
TEXT-FIG. 7 A.

Cross section through the anterior region of the olfactory organ, showing the dorsal evagination extending upward to the skin and the advanced nasal sacs at the end of the larval stage (length of larva, 48 mm.). *Cav. inf.*, cavum inferius; *Cav. med.*, cavum medium; *Cav. princ.*, cavum principale; *Dor. ev.*, dorsal evagination; *Eth. ch.*, ethmoidal part of chondrocranium; *Med. gl.*, medial nasal gland; *Mid. gl.*, middle nasal gland; *Nas. cap.*, cartilaginous nasal capsule; *Nas. sept.*, nasal septum; *Nas. duct.*, naso-lachrymal duct; *Or. ep.*, oral epithelium; *Rec. med.*, recessus medialis; *Sk.*, skin; the hatched area lateral to the dorsal evagination indicates the former external naris where the distal end of the closed entrance canal was attached.  $\times 60$ .

entrance canal. Towards the end of this stage, this entrance canal is ruptured; its proximal part becomes connected with the naso-lachrymal duct whose formation will be described later, and the distal end first appears as a peg formed beneath and then fused with the bit of skin which sealed off the former external naris (Text-fig. 6). The spot where the external naris once existed

is still recognizable up to the time of the appearance of the new naris. This new opening is median to this spot (Text-fig. 7 A).

Noble (1931, p. 62) states that in the Brevicipitidae 'the external nares do not break through until late in larval life'. In *Kaloula borealis* obviously functional external nares exist in the young larva, though they soon close up. One



TEXT-FIG. 7 B.

Cross section through the anterior region of the olfactory organ, showing the opening of the external naris (beginning of metamorphosis). *Ext.naris*, external naris; *Lat.gl.*, lateral nasal gland; *Reclat.*, recessus lateralis. Other lettering as in Text-fig. 7 A.  $\times 60$ .

wonders if other species of the Brevicipitidae may not undergo similar developmental changes resulting in having external nares at two periods in their life-history. The fact that during the major part of its larval life *Kaloula* is without external nasal opening evidently escaped the notice of Boring, Liu and Chou (1932), for in their book they mention and figure nostrils in the late-stage larva of the same species.

Recessus alaris, infundibulum, and recessus sacciformis are differentiated from the principal portion of

the dorsal evagination. The formation of these three sacs is identical with that in *Rana*, though here the process is more or less indirect. In that species these sacs arise directly from the upper part of the enlarged entrance canal. In *Kaloula* as described in a preceding paragraph, the proximal part of the entrance canal together with a part of the placode first forms a dorsal evagination. The three sacs arise in turn from it.

The formation of the *cavum principale*, *cavum medium*, *cavum inferius*, and *recessus medialis* occurs in the same manner as in *Rana*. In the absence of the ledge (see p. 308) on the lateral wall of the embryonic nasal organ, the mature *recessus lateralis* is simply derived from the side extension of the *cavum principale*.

The choana opens very widely. As soon as the upper sac together with the *cavum medium* and anterior lower sac extends anteriorly at the beginning of this stage, the choana is shifted posteriorly. Its mature position is in the ventro-posterior part of the olfactory organ—as in *Rana*.

#### B. Lateral Appendix, Accessory Glands, and Naso-lachrymal Duct.

Lateral appendix.—As in *Rana* the lateral appendix is here also an embryonic structure. Its formation, change of position and degeneration in the larval olfactory organ of *Kaloula* are, in general, similar to those found in *Rana* (Tsui, 1946b).

When the appendix reaches the height of its development (larva length, 9 mm.), it contains about three layers of epithelial cells. After this period the appendix begins to degenerate by diminishing its cell layers. This process goes on very slowly. It occupies the whole length of the second stage. In the beginning of the metamorphosis the appendix consists of only a single layer of high columnar cells. There is, however, little diminution in the size of the organ. At the end of this developmental stage its size is rapidly reduced and it becomes embedded in the olfactory epithelium as a small vestige. It disappears completely in the nasal organ of the young frog.

Glands.—The origin and development of the pharyngeal gland, and the median and lateral nasal glands are similar to

those in *Rana*. The larval lengths at the time of origin of these glands are 40 mm. for the pharyngeal gland, 17 mm. for the median, and 42 mm. for the lateral nasal gland. The first two arise later than in *Rana*, while the last one appears much earlier. Bowman's gland appears also earlier (larval length, 43 mm.). Its mouth part appears at the same time as its body, while in *Rana* the mouth appears earlier than the body. Moreover, all Bowman's glands make their appearance simultaneously in the olfactory epithelium of the *cavum principale*. In *Rana* they appear earlier in the posterior than in the anterior part of that cavity.

There is another nasal gland in *Kaloula* which is not found in *Rana* nor, to our knowledge, in any other species of *Amphibia*. It arises in the median wall of the anterior part of the upper sac above the anlage of the *cavum medium* (length of larva at the time of its appearance, 38 mm.; Text-fig. 6). It grows slowly at the second stage and at the beginning of the metamorphosis. Later, as the upper sac enlarges to form the *cavum principale*, its olfactory epithelium thickens; the gland becomes embedded in the epithelium (Text-fig. 7 A and B). At the end of this stage this gland bifurcates and increases suddenly in size. As a result of the bifurcation it possesses two ducts. In the adult stage it lies between the *cavum principale* and *cavum medium*. Its two ducts open one after the other into the ventral wall of the *cavum principale*. We shall designate it as the middle nasal gland because its position is between the median and lateral nasal glands.

**Naso-lachrymal Duct.**—As described in a preceding paragraph (see p. 304) the lumen of the distal part of the entrance canal is obliterated after the closure of the external naris. The epithelial cells at the junction of the distal end of the closed entrance canal with the skin begin to change their shape. These cells which were flat now becomes first cuboidal and then perpendicularly elongated to form a little knob below the epidermis. From this starting-point the wave of differentiation progresses in a latero-posterior direction until it reaches the lower eyelid. In this manner an epidermal ridge is formed running from the closed naris to the lower eyelid. This ridge is the

anlage of the naso-lachrymal duct. Later it becomes separated from the epidermis; the separation also starts near the closed entrance canal (Text-fig. 6) and progresses distally until the ridge is detached along its whole length. This process goes on very slowly, beginning when the larva is 38 mm. long and not being completed until the beginning of the metamorphosis.

At the time when the ridge starts to get detached from the epidermis, its loose proximal end grows medio-anteriorly for a short stretch to become connected with the proximal part of the closed and now ruptured entrance canal. That part is thus transformed into the proximal end of the future naso-lachrymal duct (Text-fig. 6). Since the entrance canal is attached to the lateral wall of the dorsal evagination at this stage, the epidermal ridge by virtue of this transformation becomes connected with it (Text-fig. 6). At the end of the second stage, the *cavum medium* enlarges and extends laterally, until the part of the dorsal evagination to which the epidermal ridge is attached becomes a portion of its lateral wall. As a result the proximal end of the future naso-lachrymal duct is now attached to the latero-posterior part of the *cavum medium*. Following this the lumen appears in the duct (Text-fig. 7 A and B). Its distal end bifurcates and the two branches open into the lower eye-lid. Thus the naso-lachrymal duct comes into being at the beginning of the metamorphosis. It undergoes no further change.

As stated above (see p. 300), in the larva of later developmental stages (24 mm. in length or over) there is a visible whitish line running from the nose to the eye. This line marks the position of the epidermal ridge. The pigment cells are crowded under the epidermis at these stages, but they are absent or scarce under the ridge, thus leaving a transparent line where the ridge lies. As a matter of fact the ridge begins to appear much earlier in life (when the larva is 14 mm. long). But at that time its presence is not easily detectable externally, because the pigment cells are then uniformly scattered under the epidermis. Under a low-power microscope, however, the ridge itself can be seen as a fine opaque line. When the ridge is completely detached from the epidermis and becomes the naso-lachrymal duct at the

beginning of metamorphosis, the epidermis thickens and the whitish line is no longer visible.

In *Rana* the naso-lachrymal duct appears much later (at the beginning of metamorphosis) and arises directly from a point in the dorso-lateral wall of the *cavum principale* behind the external naris and later, owing to transformation of the blind sacs, becomes attached to the latero-posterior part of the *cavum medium*. In *Kaloula* this duct appears earlier in larval life and arises from the epidermis of the skin. Later, as it gets connected with the lateral wall of the dorsal evagination and subsequently with *cavum medium* (the lateral wall of the dorsal evagination has become the latero-posterior wall of the *cavum medium*), the point of attachment of the naso-lachrymal duct becomes identical in the two species. Even in the point of origin of the duct, it is not so different in the two species as it appears; for the spot where the naso-lachrymal duct arises in *Rana* is actually in the former dorso-lateral wall of the entrance canal. Only, in *Kaloula* the developmental history of the naso-lachrymal duct is longer and round-about, starting in the epidermis and becoming subsequently detached.

This mode of origin of the naso-lachrymal duct in epidermis in *Kaloula* may be in some way connected with closure of the external naris. In the *Brevicipitidae* the external naris is said to open (reopen?) late in larval life. It would be interesting to know if in other species of this family the naso-lachrymal duct forms in the same way as in *Kaloula borealis*.

#### SUMMARY

1. The anlage of the olfactory placode of *Kaloula borealis* arises from the sensory layer of ectoderm as in *Rana*, but appears slightly later.

2. There is also no such structure as the oro-nasal groove.

3. There are present only the dorsal and middle lumina in the early developmental stage. A separate ventral lumen and the inward projection present in *Rana* are wanting in *Kaloula*.

4. The primitive choana opens into the endodermal part of the oral cavity as in *Rana*.



5. The external naris closes up after the formation of the primitive choana and is formed *de novo* at the beginning of the metamorphosis.

6. The floor of the middle lumen opens entirely into the oral cavity. The process which synchronizes with the closure of the external naris begins from the end next to the primitive choana and extends anteriorly. This compound opening forms the choana in the adult stage.

7. There are also three embryonic nasal sacs in *Kaloula*. Their mode of origin and prospective rôle in the formation of the mature nasal cavities are essentially identical with those in *Rana*.

8. The shrinkage of the nasal organ in metamorphosis is more pronounced than in *Rana*.

9. The lateral appendices in *Kaloula* and *Rana* are alike in their manner of formation, change of position, and degeneration.

10. The formation of the median and lateral nasal glands, pharyngeal gland, and Bowman's gland is similar to that found in *Rana*.

11. There exists a nasal gland, which is not known in *Rana*. It is designated as the middle nasal gland.

12. The naso-lachrymal duct differentiates under the skin as an epidermal ridge and is subsequently detached. It is initiated at the distal end of the closed entrance canal, but is later connected with its proximal end. When fully differentiated it opens into the latero-posterior part of the *cavum medium* at its proximal end.

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## EDWIN STEPHEN GOODRICH

1868-1946

ON January 6th of this year, 1946, our beloved and revered Professor Goodrich died at Oxford in his seventy-eighth year, scarcely three months after he had retired from his long tenure of the Linacre Chair. He is acknowledged on all hands to have been one of the great masters of Zoology. His influence will not die with his passing: his contributions to our science have been fundamental and have added so many different and vital parts to its permanent framework. As well as a great master he has been one of the great servants of Science: for a quarter of a century he has been the devoted editor of our Quarterly Journal.

Only a few weeks before he died he had corrected the proofs of the last part of that remarkable review of his 50 years' work on nephridia and coelomoducts which forms parts II, III, and IV of the previous volume. The last part was printed but not issued when he died, and before it appeared Dr. Bidder added to it the mourning notice which bore these words:

*Si monumentum quaeras, respice.*

The *Quarterly Journal of Microscopical Science* for the last fifty years is indeed his memorial; apart from his editorship since 1920, no fewer than forty-three of his research papers, from 1893 onwards, have been published in its pages.

Edwin Stephen Goodrich was born at Weston-super-Mare on June 21, 1868, the son of the Rev. Octavius Pitt Goodrich, and was the last male descendant of John Goodrich of Energlyn, Glamorganshire, who came from Nansewood, Virginia, U.S.A., in 1775. John Goodrich's forbear, also John, had gone to America in 1630 and settled in Nansewood in 1635; he in turn was descended from John, the elder brother of Thomas Goodrich who was Lord Chancellor of England, Bishop of Ely from 1534 to 1545, then Ambassador to France and again Bishop of Ely in 1551. With Professor Goodrich's death this branch of the family is now extinct.

His father having died, his mother Frances Lucinda Parker (who lived until 1936 reaching the age of 98) took the children

to the south of France at the end of the Franco-Prussian war and they settled at Pau. His elder brother returned for education to England, going to Charterhouse and Balliol, but he himself was considered too delicate and was brought up in France; he attended first a French Lycée and then an English school at Pau. So it came about that he spoke French as perfectly as English.

From his earliest years he had a taste for natural history and, inheriting a family gift for drawing, produced in his boyhood beautiful coloured studies of birds and butterflies. His keen artistic sense, as will be stressed later, was an important factor contributing to his great qualities as a teacher and master of his science; it also made him an accomplished landscape painter in water colour.

At the age of twenty Goodrich returned to England and entered the Slade School of Art at University College, London, in 1888. What a fortunate event for Zoology that was! Ray Lankester, then at the height of his powers as an inspiring teacher, was Jodrell Professor at London and lecturing in a nearby classroom; so Goodrich with his interest in natural history went to hear him. He immediately fell under his spell; he decided then and there that zoology was his real career and changed his course of studies from art to become a pupil under Lankester. At that moment a very important link in the chain of zoological history was forged. Lankester was at once greatly impressed by Goodrich's ability and when in 1891 he was appointed to the Linacre Chair at Oxford, he took Goodrich with him as his assistant. I am sure that in many Zoologists' minds the names of Lankester and Goodrich are closely linked—and with good cause. Lankester was the dominating force in the evolutionary comparative anatomy which arose and flourished after the publication of the *Origin of Species*. Goodrich more than anyone else has carried forward the torch, lit by Lankester, in this field of zoology; he has carried it from the last century up to nearly half of this and the flame burns as brightly in his last great paper just published, as it did in his early papers of the 'nineties. To say this is not to imply that Goodrich just continued lines of work started by Lankester—not at all: his genius and insight

continually opened up new lines of work bringing sense and order where hitherto had been confusion in both vertebrate and invertebrate zoology. Our Journal has of course developed and kept its fame under the guidance of these two great leaders: when Lankester gave up his long editorship in 1920, Goodrich was his natural follower.

On coming to Oxford from London Goodrich entered Merton College as an undergraduate in 1892 and while acting as Assistant to Lankester read for the final honour school in Zoology; he was awarded the Rolleston Memorial Prize in 1894 and graduated with First-class Honours the following year.

An account of his scientific work will come later, but while we are being biographical it should be recorded that when still an undergraduate he had become a most active researcher. He was of course somewhat older than the average undergraduate; he was twenty-four when he entered Merton and in that year he published his first two papers. His third paper, and his first in this Journal, came in 1893, two more in 1894, and before he took his final schools he must have completed the two papers published in 1895 which included his ever famous dissertation 'On the Coelom, Genital Ducts and Nephridia'. His wide range of interest was already clearly shown: apart from this classic, which covered the whole range of the Metazoa, his early papers had dealt with cephalopods, polychaetes, oligochaetes, fossil mammals and museum reform.

After graduation he went with the Naples Scholarship for six months to the famous Stazione Zoologica and in 1898 he was awarded the Radcliffe Travelling Fellowship, whereby he visited India and Ceylon. In the following year he was appointed Aldrichian Demonstrator of Comparative Anatomy and in 1900 was elected a Fellow of Merton. Apart from his world-wide travels in vacations Goodrich remained at Oxford all his life. W. F. R. Weldon had become Linacre Professor in 1899 when Lankester went to be Director of the Natural History departments of the British Museum and was in turn succeeded in the chair by G. C. Bourne in 1906. During the first world war when Bourne and other members of the staff were away on war service, Goodrich carried on the teaching and administration of the

department single-handed. A special Professorship of Comparative Embryology was made for him in 1919, and in 1921 he succeeded Bourne in the Linacre Chair, which he held until last year. All this time, until his death Goodrich remained a Fellow of Merton, becoming a Professorial Fellow when he took the Chair, and being elected an Honorary Fellow when he retired.

In 1913 he married Helen Pixell, the eminent protozoologist, and more will be said of this very happy partnership later. Here I want to refer to something that is so characteristic of his modesty: he would not take his D.Sc. degree until some years after his wife had taken hers and then only when persuaded to the extent of her paying the necessary University dues and providing the gorgeous and costly robes!

Many were the honours that came to him. He was elected a Fellow of the Royal Society in 1905, when still in his thirties, served on the Council twice (1923-5 and 1931-2) and as Vice-President during 1930-1, and was awarded the Royal Medal in 1936. From 1915 to 1923 he was Zoological Secretary of the Linnean Society of London, helping to keep it alive through those lean and difficult years of war; in 1932 he received the Society's Gold Medal. He was given an Honorary LL.D. by Edinburgh University and an Honorary Sc.D. by Dublin. He was Hon. Member of the New York Academy of Sciences, Member of the Royal Swedish Academy, Membre Correspondant de la Société de Biologie de Paris, Associé de l'Académie Royale de Belgique, Foreign Member of the Academy of Sciences of U.S.S.R., Leningrad, Hon. Fellow of the National Institute of Sciences of India, Member of the International Institute of Embryology of Utrecht and Member of the Royal Society of Sciences of Upsala.

On his seventieth birthday, in 1938, his colleagues and pupils expressed their admiration for his work by presenting him with a congratulatory volume of essays edited by Dr. (now Professor) G. R. de Beer and entitled *Evolution: Essays on Aspects of Evolutionary Biology*.

With this brief biographical summary let us now turn to consider his contributions to zoological knowledge and his influence as a teacher. Appended to this account of his work will be found

a complete list of his publications arranged in chronological order, which was kindly prepared for me by Mrs. Goodrich; to her also I am much indebted for notes regarding his career.

Goodrich's first paper, published in the *Journal of the Marine Biological Association* in 1892 was a precise account of a large and rare squid *Ommastrephes pteropus* Stp. which had been captured off Salcombe; but he was not content with presenting just a careful description, for he added to it a valuable table giving the chief characters of all the genera of recent Oigopsid Cephalopoda in the form of a key. Four years later, in his tenth publication, he wrote a report on a collection of 162 Cephalopoda belonging to 28 genera, collected by H.M.S. Investigator in the Indian Ocean; he described and figured eleven entirely new species belonging to nine genera and recorded four genera not hitherto taken in the Indian region.

In 1892 he also published a note on a new species and genus of oligochaete *Vermiculus pilosus*, which he discovered at Weymouth, and in 1895 gave a full account of it in this Journal; he showed that it has a number of characters which place it in a very isolated position including a dense covering, from head to tail, of remarkable 'sense hairs'. He wrote further notes on oligochaetes with a description of a new species *Enchytraeus hortensis* in 1896, here paying particular attention to the coelomic corpuscles.

As soon as he came to Oxford he began to assist Lankester in an entire rearrangement of the zoological collections in the University Museum. He described these reforms in the now extinct monthly journal *Natural Science* in 1894. It is clear from his writing how enthusiastically he took up this task.

'Here', he writes, 'one need seek neither to attract the nursery-maid nor to amuse children, nor again need one trouble to satisfy the idle curiosity of the sightseer. There is, then, no necessity for tragic groups of stuffed animals, for birds perched on cardboard rocks among artificial flowers. On the contrary, the exhibits are to be strictly scientific, forming series at once instructive and interesting to the general educated public, and more especially to the real student of

zoology. Surrounded as it is by the various chemical, physical, and biological laboratories, the central court is in the first instance a place of study. In such educational collections it is essential that each object should be exhibited for a definite purpose, should show what it is meant to show as clearly as possible, and should be fully labelled in language technical so far as is necessary for accuracy. The observer is not to be bewildered by a number of specimens, but rather impressed by a few well-chosen examples.'

He was responsible for the greater part of the vertebrate exhibits. As a Professor from another University remarked to me recently 'there is no other museum in which a student can learn so much sound comparative anatomy by walking round and studying the exhibits and their labels—thanks to Goodrich'.

This rearrangement of the museum led to Goodrich's first published work in palaeontology: his paper (1894) on the fossil Mammalia of the Stonesfield Slate. How characteristic of him is the manner in which he came to undertake it. In this paper he writes: 'Through the kindness of Professor Green and Professor Lankester, who placed the Oxford fossils in my hands for the purpose of displaying them in a museum case in a manner more worthy of their interest and value, I had the opportunity of examining and handling our six specimens.' These were the famous lower jaws of the first Mesozoic mammals to be discovered; they had been found about 1814 and examined and described by a succession of authors including Buckland, Cuvier, Owen and Osborn. Their particular interest apart from their early origin lay in the nature of their teeth and the light they shed on the evolution of mammalian dentition. Goodrich was not content simply to display the specimens with explanatory labels setting out the current theories concerning them; he must thoroughly re-examine them himself to see if they really did support the theories. We must remember that at this time he was still an undergraduate working for his schools. He also obtained access to the three other known English specimens: two in the British Museum and one at York. He states the 'Tritubercular Theory' advanced at that time by Osborn, Cope

and other American palaeontologists and then characteristically writes: 'Let us now examine the facts.'

All through his life's work that phrase, or some other like it, recurs again and again; he states the generally accepted theory and then examines the facts: the actual specimens concerned. So often, as here, he proved the theory false, or else greatly enhanced its value. The then widely accepted American view was that the Tritubercular tooth, with cusps arranged in a triangle, was derived from a Triconodont type, with the three cusps in line, by the shifting outwards of the median cusp; the Triconodont condition was in turn considered the first step towards the more complex mammalian molar by being derived from the simple reptilian cone by the addition of an extra cusp in front and behind. 'Professor Osborn', writes Goodrich, 'in his illustrations of the theory . . . has made large use of the Mesozoic mammals found in England; one can therefore stand on firm ground while criticising his conclusions and his interpretations of the facts.' By carefully working away the matrix Goodrich exposed new cusps to some of the teeth and in some cases new teeth. His re-examination demolished the supposed evidence upon which Osborn's theory was based and led him to conclude 'that the common ancestor of Marsupial and Placental mammals had teeth with many cusps of the Tritubercular sectorial pattern' and that it 'seems extremely probable' that the molars of the earliest ancestral mammal 'were of an indefinite multituberculate pattern'.

In the meantime he had in 1893 published his discovery of the dorsal ciliated organ in *Nereis* and found it in other closely allied polychaetes but not in genera of other families; he states his reasons for considering it 'as a genital duct not fully developed'. He also gives a beautiful description of the nephridium of *Nereis*. This is the beginning of that magnificent series of studies on nephridia and coelomoducts to which he returned and added to so often throughout his life until the very end. When he wrote this, his first contribution to the subject, it was generally believed that genital ducts and nephridia were homologous structures; here he points out the difficulties of this view and with remarkable insight tentatively foreshadows the



conclusions which he was subsequently to establish beyond doubt.

Two years later, in 1895, came his classic 'On the Coelom, Genital Ducts and Nephridia'. It is written with his typical lucidity and beautiful economy of words; in a text of only twenty-six pages, in which he refers to 116 other published researches, he compares all the main groups of the triploblastic Metazoa and shows the thread of homology running through them. The significance of this paper cannot be better expressed than in his own words taken from its introduction:

'An unprejudiced review of the well-established and recently ascertained facts concerning the development of the excretory organs and genital ducts of the Coelomata must, I think, inevitably lead us to the conclusion that we have been confusing two organs of totally different origin under the one name nephridium—the one organ the true nephridium, the other the morphological representative of the genital duct, which may be called the peritoneal funnel, to avoid confusion. Further, that while on the one hand in certain groups such as the Planaria, Nemertina, Hirudinea, Chaetopoda, Rotifera, Entoprocta, besides the genital ducts or peritoneal funnels, we find true nephridia in the adult; on the other hand, in such groups as the Mollusca, Arthropoda, Ectoprocta, Echinoderma, and Vertebrata, there are in the adult no certain traces of true nephridia. In these latter groups, as we shall see, the peritoneal funnels (primitive genital ducts) take on the excretory functions of the nephridia which they supersede.'

It will be remembered that at this time Goodrich had not yet discovered the nephridial nature of the excretory organs of *Amphioxus*.

He continues:

'In the following brief review of the various classes of Coelomata, I shall endeavour to show that the two kinds of organs can always be distinguished; that the first, the nephridium, is primitively excretory in function, is developed centripetally as it were, and quite independently of the coelom (indeed, is probably derived from the epiblast), possesses a

lumen which is developed as the hollowing out of the nephridial cells, and is generally of an intracellular character, is closed within, and may secondarily acquire an internal opening either into a blood space or into the coelom (true nephridial funnel as opposed to the peritoneal funnel); and that the second kind of organ, the peritoneal funnel, is primitively the outlet for the genital products, is unvariably developed centrifugally as an outgrowth from the coelomic epithelium or wall of the genital follicle, is therefore of undoubtedly mesoblastic origin, and possesses a lumen arising as an extension of the coelom itself.

‘In the series of diagrams illustrating this paper, based on the most recent and accurate researches, it has been my constant endeavour to interpret the author’s results correctly and not to distort the facts in favour of the theory here advocated.’

He then proceeds to compare the conditions found in the different coelomate Metazoa group by group.

In the following year (1896) he replies in the *Zoologischer Anzeiger* to a critical review of his paper by Bergh; in this he makes a dramatic reference to his discovery of solenocytes which he had not yet announced.

‘The reviewer’, he writes, ‘appears still to hold the view that the nephridia of the Oligochaeta are homologous not with the nephridia (excretory organs) of the Platyhelminths and Nemertines, but with the follicle-ducts of the latter; I contend that recently ascertained facts concerning the anatomy and development of these organs render the theory untenable. It may be said that if the theory which I on the other hand advocate be true, I should be able to show an Annelid with an undoubted coelom into which the “true nephridia” do not open—to this I can answer, that I believe I am now in a position to supply this long sought link in the chain of argument (*Nephthys* and *Glycera*: as I hope to show in a forthcoming paper).’

There then followed that remarkable series of papers ‘On the Nephridia of the Polychaeta’, Part I in 1897 dealing with

Hesione, Tyrrhena and Nephthys, Part II in 1898 on Glycera and Goniada, and Part III in 1900 dealing with the Phyllodoctidae, Syllidae, Amphinomidae, &c., and general conclusions. Here he describes and figures with superb drawings the minute and delicate anatomy of the excretory and genital ducts of so many different polychaetes. Here are his discoveries of the solenocytes of various kinds, the blind endings of excretory canals comparable to the flame cells of the Platyhelminths but differing from them in the possession of fine straight tubes, enclosing the flagellum, running from the cells to the lumen of the canal. Here he also describes the different conditions of combination of nephridium and genital duct or funnel that he calls the nephromixium.

There is no need here to stress the immense amount of work he has described in these and subsequent papers; as already mentioned and as all will know, he had so recently completed, just before he died, that magnificent review of all the work done in this field since the publication of his paper of 1895. It is written with the same economy of words, yet instead of twenty-six it has 266 pages and refers to 430 other papers. It is more than a review of past work for it contains many original and hitherto unpublished observations; the most important of these is perhaps his study of the early stages of the nephridioblast in *Tubifex* where he clearly shows that it was never a cell derived from the coelomic epithelium as believed by Meyer. His own work and that of others have fully confirmed the conclusions he formerly reached regarding the nature of the two kinds of ducts; in addition the gonocoel theory is accepted as the best explanation of the origin of the coelom. Professor de Beer in his Royal Society obituary of Goodrich has well said: 'Even if he had done nothing else this last paper would be sufficient to ensure his lasting reputation as a zoologist.'

In spite of this so recent review of his, we must not leave the subject of nephridia without recalling what is perhaps his most exciting discovery: the solenocytes of *Amphioxus*. In 1890 both Weiss and Boveri had independently discovered the excretory organs of *Amphioxus* but had described them as tubules opening into the coelomic cavities; they took them to be the primitive

homologues of the pronephric tubules of the higher chordates. Goodrich in his third paper on the nephridia of the Polychaeta (1900) records in a footnote how he was struck by the 'strange resemblance between the solenocytes' (which he had discovered in the Polychaetes) 'and the peculiar cells described by Boveri as surrounding the openings of the excretory tubules in *Amphioxus*'. He goes on to say: 'Some years ago, I examined these tubules in fresh specimens and came to the conclusion that the resemblance is only superficial.' The following winter, however, when working at Naples, he decided to re-examine them and was now able to show that they were indeed true solenocytes and there was in fact no funnel opening into the coelom at all. They were not homologous with the kidney tubules of vertebrates which he had shown to be coelomoducts but were true nephridia and bore a remarkable resemblance to the solenocytes he had described in *Phyllodoce*. It must have given him great satisfaction to have had the opportunity of personally demonstrating the correctness of his observations to Professor Boveri who happened to be visiting Naples at the time.

As will be seen from his list of publications he returned again and again to the study of *Amphioxus*; he made many journeys to obtain material to Sicily, Naples, Heligoland and Bermuda. His proficiency in the Italian language, as well as French, was a great help to him in his field work, for he was often out with the fishermen in their small boats going from Messina to Cape Faro at night or very early in the morning in search of *Amphioxus*. Great was his disappointment to find that the plentiful supply there earlier in the century had been exterminated by the earthquakes of 1912, probably by a wave of hot water, and had not been replenished twelve years or more afterwards. Overcoming the many difficulties, technical and otherwise, he produced further detailed studies on the nephridia of *Amphioxus* and their development in 1909, 1932, and 1934 as well as a short note on 'Hermaphroditism in *Amphioxus*', 1912, and a paper on the development of the club-shaped gland in 1930. Other beautiful studies of solenocytes in larval forms must just be mentioned: his account of the body cavities and nephridia of the *Actinotrocha*

larva of Phoronis (1903) and the nephridia of the larvae of Echiurus and Polygordius (1909).

He wrote two papers on *Sternaspis* (1898 and 1904) which, although originating in an examination of the genital organs and nephridia should be mentioned for their fine anatomical studies of the muscular and vascular systems. I cannot resist quoting from the first of these papers for quite another reason: it illustrates so well his characteristic combination of courtesy and directness in demolishing the false views of others. He begins the paper thus: 'In the beautiful works both of Professor Vejdovsky and M. Rietsch on *Sternaspis* we find certain statements which, if correct, would place that worm in a very exceptional position.' He then briefly describes the statements concerned and goes on: 'It was, therefore, with a view to either confirm or correct these descriptions that I began a study of *Sternaspis thalassemoides*, Otto, during a recent visit to Naples. I may say at once that they both proved to be erroneous.' His clear interpretations and drawings of his serial sections then supply the proof.

In 1898 with his remarkable morphological insight he cleared away much previous confusion in his essay on the segmentation of the Arthropod head and his thesis has well stood the test of time. His arguments in favour of there being six segments in the Crustacean head were largely based on evidence from the nervous system, for at that time distinct somites or coelomic cavities had not yet been traced with certainty in the development of the cephalic region. How pleased he must have been when Miss Manton in 1928 in her beautiful embryological study of *Hemimysis* clearly demonstrated six mesoblastic somites in the head.

To group after group of invertebrates he applied his skill; in the same year he solved a nice problem in the Mollusca. Again I cannot resist giving a quotation:

'Strange indeed, and happily unique in the annals of comparative anatomy, has been the history of our knowledge of the reno-pericardial canals of *Patella*. Although discovered more than thirty years ago, and investigated by many

observers since, not only is their structure insufficiently known, but their very existence has been called in question and even positively denied!

'Wishing to find out definitely whether these ducts really existed or not, I undertook this work, which was carried out in Oxford, on material obtained from Plymouth and Naples. In this short paper I hope to establish clearly, and beyond the possibility of doubt, the fact that there are reno-pericardial canals leading from the pericardium to the right kidney and to the left kidney in *Patella*.'

And he did.

In the following year, 1899, he made a study of the coelom and vascular system of the Leech and in 1900 came his section on the Holothuroidea in the Echinoderm volume of Lankester's *Treatise on Zoology*.

Space will not permit more than a passing reference to his keen interest in the Archiannelids and the problem of their systematic position: whether they are to be regarded as primitive or secondarily simplified; he has studies on *Saccocirrus* (1901), *Dinophilus* (1909), *Nerilla* (1912), and *Protodrilus* (1921). In the *Nerilla* paper he sums up his conclusions: 'Taken as a whole the Archiannelida form a degenerating series which can only be read one way. But very possibly the group includes three such series starting from a common Chaetopod ancestor, *Chaetogordius* and *Polygordius* forming one, *Saccocirrus* and *Protodrilus* another, and *Nerilla*, *Dinophilus* and *Histriobdella* a third.' He also wrote three papers on new or little known Syllid worms (1900, 1930 and 1933).

In a paper in 1919 he showed that the slender pseudopodia usually described and figured as projecting from the leucocytes of invertebrates are in reality the radial folds of an extensive membrane which surrounds such cells and had hitherto escaped detection. In the same year he and Mrs. Goodrich collaborated in a most interesting study of the ecological interrelationships between leucocytes and parasitic protozoa, bringing out among other things the conclusion that most such protozoa must produce some secretion which causes leucocytes to avoid them;

together they also described (1920) a new species of Gregarine, *Gonospora minchinii*, inhabiting the egg of *Arenicola*. Here too may be a convenient place to refer to one of his very recent papers, 1942, on a new method of dissociating cells: by immersing small pieces of tissue, or whole small animals such as *Hydra*, in a saturated solution of boric acid in normal salt solution to which a trace of Lugol's solution of iodine has been added. The cells fall apart, or may be easily separated, retaining their characteristic form; he shows many beautiful examples such as ecto- and endodermal musculo-epithelial cells of *Hydra*, cells of the intestine and the nephrostome of *Lumbricus*, cells from various tissues of the frog and rabbit: all most valuable for class work.

During the recent war the importance of studying and controlling the insect pests infesting grain and other stored products has been fully realized and many have been engaged in such researches. In the previous war Goodrich was a pioneer in this field; he sought to find out how the presence of parasitic Hymenoptera (Chalcidae) may affect the various grain-infesting beetles. First he showed that some species of beetle were parasitised and others not, and that those which were, were attacked in the larval stage. He then showed that the Chalcids could not be effectively used in keeping down these beetles because, as he discovered, they themselves were in turn parasitised and kept in check by an acarid *Pediculoides ventricosus* Newport which had not hitherto been known to attack hymenoptera. Rarely in an official report (1921) do we see such a vivid description as he gives:

'In a grain of wheat are often found the shrivelled remains of the *Calandra* larva on which the hymenopteron larva has fed, the dead or dying Chalcid imago, and the *Pediculoides* attached to it. Thus the whole series of events is permanently recorded in chitin, and the complete tragedy can be unfolded, even from unpromising material, by soaking it in a strong solution of potash.'

Up to here, the only vertebrate work of his we have noted is his study of the fossil mammals of the Stonesfield Slate. If a

novice in Zoology should read this article thus far, he would get the impression that Goodrich was mainly an invertebrate specialist; we must now correct that impression. Great as have been his contributions to invertebrate Zoology, still greater are his achievements among the vertebrates.

In 1901 in making a study of the pelvic girdle and fin of the fossil fish *Eusthenopteron* he came to compare the pelvic girdles and fins of all groups of fish. There was at that time considerable confusion regarding the morphology of the pelvic supports; it was commonly held for example that a true pelvic girdle was present in the *Selachii*, *Holocephali* and *Dipnoi* but that the supports in the *Crossopterygii* and *Actinopterygii* were derived from the fin skeleton itself. 'Let us see what difficulties such views lead us into', he says, and at once proceeds to one of his masterly analyses of the different theories side by side with a study of the actual specimens concerned. The logic of his argument inevitably leads us to the 'conclusion that the pelvic supports, whether paired or unpaired, are homologous throughout the fish series'. He now became greatly interested in the fins of fish and the importance of their differences in structure in classification. In 1903 he published his beautiful studies of the dermal fin rays of both living and fossil forms. Having clearly distinguished the four different kinds: the *ceratotrichia* of the *Elasmobranchii* and *Holocephali*, the *actinotrichia* and *lepidotrichia* of *Teleostomes* and the *camptotrichia* of the *Dipnoi*, he then proceeds to discuss their origin and homologies. He shows that the *lepidotrichia* are of quite a different nature from the horny *ceratotrichia* and *actinotrichia* and are undoubtedly derived from modified body scales. Further, although not conclusively proved, he shows it likely that the *camptotrichia* of the *Dipnoi* are homologous with the *lepidotrichia* of the *Teleostomes* but have sunk deeper and been overlaid by a secondary extension of the body scales.

The two foregoing papers, important in clearing up much confusion, were but the prelude to his grand attack on the problem of the origin and nature of the paired fins in 1906. It was a major problem of the time, for there was little doubt that the paired fins of fish and the limbs of the higher vertebrates were



homologous. Zoologists were divided into two camps: those who followed Gegenbaur in believing the paired fins to be derived from gill structures (the gill-arch theory) and those who followed Balfour, Thacker and Mivart in the view that they were derived from paired longitudinal fin folds of a similar nature to the median fins (the lateral fin-fold theory). 'Each of these theories', he says, 'may claim to have among its numerous supporters the names of some of the most eminent exponents of the morphology of the vertebrates.'

The literature on the subject had been extensive, but it practically came to an end when Goodrich stepped in and settled the matter for good and all. It is impossible in a brief review to do justice to his case, but let us just remind ourselves of some of the more telling points he made in this which was another of his classic papers. The paired fins develop on the whole just like median fins: the muscle buds grow out from the myotomes, divide into upper and lower halves to supply each side of the radials which are differentiated between them. There is a remarkable resemblance in detail of structure between the paired and unpaired fins. If the paired fins had developed from vertical gill septa they would in the first instance have hindered forward locomotion and the two pairs would have been close together one behind the other and so mechanically ineffective. In development the fin never appears as a dorso-ventral fold, but always as a longitudinal one. There is no evidence, either from primitive living fish or early fossil forms, of a more anterior pelvic fin which might be expected if it was derived from a gill arch; in those Teleosts where the pelvic fin is far forward, there is good evidence that this is a recent and secondary development. The presence of rudimentary muscle buds in front of the pelvic fins had been supposed to indicate a backward migration of the fin from a primitively more forward position; Goodrich, however, showed that rudimentary muscle buds may also be found *behind the fin*. The gill-arch theory does not account for the large number of segments often contributing to the muscle buds of the fins and the fact that usually more segments are concerned in the more primitive forms. And then the *coup de grâce*: the gill arches are morphologically in the wall of the ali-

mentary canal and are supplied by visceral muscles innervated here by *dorsal roots* (vagus) whereas the paired fin muscles are derived from myotomes and innervated by somatic motor nerves from the *ventral roots*.

This same paper did much more than clear up the question of the paired fins; it dealt also with the development, structure and origin of the median fins. He showed how the 'concentration' of muscle buds and radials found in the fins (both median and paired) came about: the muscle buds having been nipped off from the myotomes, the body of the fish now grows faster than the fin so that the series of muscle buds no longer corresponds in length with the series of myotomes which gave rise to the buds but appears as a concentration.

His studies of the nerve supply of the muscle buds of fins and limbs led him to enunciate a general principle which is of great importance in helping the comparative anatomist in the correct interpretations of evolutionary morphological problems: that the motor nerves always remain faithful to their particular myotomes or their derivatives even, as he says 'throughout the vicissitudes of phylogenetic and ontogenetic modification'. It seemed to him, both on physiological and anatomical grounds, highly improbable that a motor nerve could forsake the muscle in connection with which it was originally developed to become attached to another muscle of different origin. All his work on the development of the limbs supported this. They are supplied by branches from a number of segmental nerves forming a plexus, but such a plexus can be shown to be 'brought about, not by the nerve deserting one muscle for the sake of another, but by the combination of muscles derived from neighbouring segments'. He tested this experimentally in the living skate by observing the separate contraction of the different muscle elements of the plexus when their corresponding nerves were stimulated by electrical and mechanical means.

All through Goodrich's work we see his curiosity being aroused by points which set him off on fresh lines of fruitful investigation; he had a remarkable gift for picking out problems of importance, the significance of which had been missed by others. We now come to the puzzle of *Polypterus*. In his paper

of 1901 on the pelvic fins and girdles he draws attention to the position of this fish which for so long had been placed with the Crossopterygii and hints that he considers it not unlikely that it is really an Actinopterygian. In 1907 he read a paper to the Zoological section of the British Association on 'The Systematic Position of Polypterus' in which he develops the thesis more definitely. It had been placed in the Crossopterygii by Huxley on account of its lobate paired fins, paired gulars, rhomboid scales and outwardly diphyccercal tail. Goodrich had already shown that internally its paired fins were of quite a different structure from those of the Crossopterygians to which they had but a superficial resemblance. He now showed the same thing for the scales, the two kinds look alike but those of Polypterus are not covered with cosmine but are of a true ganoid type resembling closely those of the fossil Palaeoniscoids. The paired gular plates might just as well be compared with the anterior members of the lateral series of plates of an Actinopterygian (such as the branchiostegal rays of *Amia*) as with the more median pair of gular plates of the Crossopterygians—indeed he pointed out that among the fossil Actinopterygians there are the Palaeoniscidae which in fact have just such anterior lateral plates enlarged. Again internally the caudal fin of Polypterus shows evidence of being a modified heterocercal tail. He now definitely believed it should be regarded as an Actinopterygian—but he was cautious about going further at that time, although reading between the lines one can sense his leaning to the belief that it is really a living Palaeoniscid. In his great book on the Cyclostomes and Fishes published in 1909 he still places Polypterus among the Crossopterygians but at their end, next to the Actinopterygians, and indicates his belief that they will be proved to belong to the latter; here however we see a certain caution owing to his having seen the resemblance of Polypterus to *Tarrasius problematicus* which was then regarded as an Osteolepidotid Crossopterygian. But I must cut a long, and exciting, story short. It was not until 1927 that Goodrich published his 'Polypterus a Palaeoniscid?' in which he fully gives the reasons with which he had 'ventured to suggest that the Polypterini are survivors of this large and varied group

hitherto supposed to be extinct'. His brilliant pupil, the late Mr. J. A. Moy-Thomas, now put the finishing touches to the story; his tragic death in the war, such a great loss to Zoology, was a personal grief which Goodrich felt very deeply.<sup>1</sup> Moy-Thomas published his study<sup>2</sup> of the development of the chondrocranium of *Polypterus* in 1933 and compared it with the development of the chondrocrania of other fish; he ended by saying 'the view of Goodrich is thus afforded additional support'. In the following year he, Moy-Thomas, proved that *Tarrasius problematicus* was actually a Palaeoniscid.<sup>3</sup> Goodrich's suspicions were confirmed in both directions: *Polypterus* resembled both *Tarrasius* and the Palaeoniscids for the now simple reason that the two were shown to be of one and the same Actinopterygian group.

In 1939 Moy-Thomas in his book *Palaeozoic Fishes* (p. 117) definitely refers to '*Polypterus*, itself a palaeoniscid derivative' and ends his book with these words:

'The African *Polypterus* is probably directly descended from the Palaeoniscids in the Cretaceous, and has retained the Palaeoniscoid scales, but has become rather specialized in other ways, especially in the nature of its fins.'

The puzzle of *Polypterus*, indeed an exciting story of detection, is ended. We have here a living fossil, almost as remarkable as the living *Coelocanth Latimeria*, discovered not, as was the latter, by its sudden appearance in a trawl, but by the methods of comparative anatomy of which Goodrich was the master: the comparison of both living and fossil forms together.

Again one thing leads to another. Both his work on the dermal fin-rays of fish and his interest in the scales of *Polypterus* led him on to another of his major contributions to vertebrate morphology: his study 'On the Scales of Fish, Living and Extinct, and their importance in Classification', published in 1908. Here he gave us his beautiful drawings of sections of the

<sup>1</sup> See his obituary notice of Moy-Thomas in *Nature*, April 8, 1944.

<sup>2</sup> *Q.J.M.S.*, 76. 209.

<sup>3</sup> *Proc. Zool. Soc. London*, 1934, 367.

different kinds of scale and his interpretations of them. Williamson half a century earlier had shown that some of the so-called ganoid scales of Agassiz, those of fossil osteolepidotid fish such as *Megalichthys* were of what he called a cosmoid type, formed by a layer of fused denticles—the cosmine layer—becoming attached to an underlying bony plate, the so-called ‘isopedin’ layer. Goodrich largely confirmed Williamson’s findings and called attention to his somewhat neglected work which had been hidden by the more recent and, as Goodrich showed, erroneous theories of Oscar Hertwig. He distinguished the cosmoid scales of Williamson from the true ganoid scales and further subdivided the latter into those of the Palaeoniscoid type, found in the fossil Palaeoniscids and the living *Polypterus*, and those of the Lepidosteoid type found in *Lepidosteus* (and the *Amioidei*). The former he showed to be evolved from the cosmoid type by the addition of layers of ganoin on top: a sandwich of cosmine between the ganoin and bony isopedin plates. The Lepidosteoid scale he showed to be similar but lacking the intermediate layer. He went on to demonstrate the great value of these different scales as an aid to classification.

This work in turn led to an interesting discovery he published in 1913 but which is best mentioned here: one concerning the structure of bone in fishes; a ‘contribution to palaeohistology’ he called it. In addition to the differences between the palaeoniscoid and lepidosteoid scales just referred to, it was now found that the bony layers of the latter were quite different from those of the former; in the lepidosteoid scale the layers of bone are traversed at right angles by peculiar tubules which in the living tissues are filled with the long protoplasmic processes of large cells situated on the surface of the scale. He now discovered that this condition was also present not only in those dermal bones of the skull originally derived from scales but in the *whole endoskeleton* as well, that is of those Actinopterygian fish which have Lepidosteoid scales (the *Amioidei* and *Lepidosteoidei*) and in those fish alone. He gives a list of the fish of many groups he has investigated. ‘It follows that, from the examination of the minutest fragment of the skeleton of a living or extinct species of fish we can decide whether it belongs to these two orders or

to some other group. The histological structure of the bone may therefore be of the greatest practical value for the identification of fragmentary specimens.' He had placed a new tool in the hands of the phylogenetic 'detective'.

In 1909 came his *magnum opus* on the 'Vertebrate Craniata: Cyclostomes and Fishes' forming Part IX of Lankester's *Treatise on Zoology*. All will know that it has been, and will continue to be for very many years, the standard text-book on the morphology of fish. While a literature of over 500 items is referred to, it is no mere compilation of the work of others; it is threaded through and through with his original observations on both recent and fossil forms, and the majority of the observations of others have been checked by him personally. Here, in addition to the results of his researches on scales and fins already referred to, are his masterly accounts of the skull and axial skeleton, the segmentation of the head, the nerve components, the nature and development of the coelomoducal kidneys, the vascular system and the air bladder; this is to mention only some of the many subjects all treated from the evolutionary point of view and clarified by his peculiar morphological insight. More than 150 of the illustrations are original, and many of them are those semi-diagrammatic but nevertheless accurate figures showing the three dimensions of space which are such a godsend to the student. Flesh and fossil, Science and Art have rarely been combined as here. It is superfluous to say more when the fame of the book is world-wide; I will just add a quotation from an appreciation of Goodrich by Dr. Julian Huxley in *The Times*:

'As an example of the international esteem in which he was held, I should like to record what Professor Berg, the leading Russian authority on fishes, said to me in Leningrad this summer, in asking me to take charge of a book for presentation to Goodrich: "Please tell him that, though neither I nor my colleagues have ever met him, we all regard ourselves as his pupils."'

Following a laudatory review of this book, 'W. E. A.' in *Nature*, 1909 (vol. 82, p. 152), among a very few 'points of minor importance which call for criticism', writes:

'On p. 116 we read, as one of the *primitive* characters of the Pisces (which group here does not include the Cyclostomes), that the pericardium may communicate with the abdominal coelome. In view of the fact that this communication in Elasmobranchs is formed secondarily in ontogeny after the two cavities have been completely separated from each other, it would have been better not to have included it in the list of characters "considered primitive" without a qualifying note.'

I mention this 'minor point' because it has an interesting outcome. Goodrich did not reply for nine years, not until he had characteristically had an opportunity of re-examining the facts, and when he did he made no reference to 'W. E. A.'s' criticism. In 1918 he published his study, with some of his best three-dimensional reconstructions of sections, 'On the Development of the Pericardiaco-peritoneal Canals in the Selachians'. I quote the two opening sentences of his summary:

'Balfour's suggestion that the canal leading in the adult Selachii from the pericardial to the peritoneal coelom, and opening into the latter by paired apertures, is a remnant of the wide communication between these cavities in the embryo is correct. The canal openings are not new formations as Hochstetter maintained, but are derived from the pericardiaco-peritoneal passages above the mesocardia lateralia.'

In 1910 he took part, with the other leading zoologists of the day, in the famous two days' debate, recorded in the *Proceedings of the Linnean Society*, on Gaskell's theory of the origin of the Vertebrates from Arthropod ancestors, which of course he opposed. The Vertebrates cannot be descended both from a form like *Amphioxus* and from an Arthropod; the supporters of Gaskell's heterodox views regarded *Amphioxus* not as a primitive form but as a secondarily simplified degenerate vertebrate. Although Gaskell's theory, that fascinating but gigantic folly of phylogenetic speculation, appears to-day to be dead and forgotten, there may still be a misguided few who prefer to regard *Amphioxus* as degenerate rather than primitive. If there

are, let them for a moment listen to Goodrich making some of his telling points in the debate:

‘Now, although *Amphioxus* is doubtless in some respects a very specialized animal—as for instance in the possession of an atrial cavity—yet it preserves many primitive characters. Judging from its structure, we must conclude that the ancestral Vertebrate was still more uniformly segmented than the primitive Craniate. The head-region was scarcely differentiated at all, there was no skull (probably no cartilaginous axial skeleton at all), a quite rudimentary brain, no specialized cranial nerves, no cephalization due to the presence of large paired organs of sense. It is possible that *Amphioxus* is somewhat degenerate; but it cannot seriously be urged that it once possessed in well-developed condition those paired sense-organs which have so profoundly modified the structure of the head-region in the Craniata. For it would be ridiculous to suppose that the modified segments could be restored to their original condition of uniformity with the trunk segments; no trace of the disturbance appearing in either adult or embryo.

‘Further, in *Amphioxus*, there is no dermal or epidermal armour, and primitiveness is shown in the structure of the endostyle, which becomes modified into the thyroid gland in higher forms. Lastly the presence of true nephridia, a type of excretory organ which has been lost in other Vertebrates, links *Amphioxus* to the lower Invertebrate Coelomata.

‘Thus can be traced an irreversible series of stages in the differentiation of Vertebrate structure, at the bottom of which we find a much simpler, but still essentially Vertebrate ancestor, probably already extinct in Silurian times.’

In the 1906 paper on the development of fins Goodrich first pointed out a most important conclusion that I have not hitherto referred to; I have delayed mention of it because it was in two later papers, in 1911 and 1913 that he developed these conclusions to their full and surprising significance. In 1906 he had shown that in the paired fins, as in the median ones, different series of segments were involved in different species of fish. In



the course of evolution there had been a change in the position of the fins up and down the body; this he showed was not due to an actual migration of the fin material itself, but was brought about by the incorporation of fresh segments to the front of the fin and a reduction of those taking part behind or vice versa: so producing an apparent 'migration'. In 1911 in his paper 'On the Segmentation of the Occipital Region of the Head in the Batrachia Urodela' he showed the same thing taking place in the hind region of the head. The problem he tackled had arisen thus. The occipital region of the Amniota includes behind the vagus nerve four scleromeres enclosing three roots of the hypoglossal nerve, thus making at least five segments between the auditory capsule and the Atlas; in the fish the post auditory region while less definite always includes at least seven segments; but in the Batrachia the skull appears to end immediately behind the vagus foramen. He writes thus:

'These facts immediately suggest several questions:—Does the occipital region of the Amphibian really include fewer segments than that of the other Gnathostomes, or have certain segments been telescoped and practically crushed out? Are the hypoglossal segments of the Gnathostomes really represented by the first three trunk-segments of the Amphibian, or have these simply assumed the function originally fulfilled by others farther forward? Further, if the Amphibian head includes fewer segments, it may be asked whether this condition is primary, or due to the return of segments to the trunk which formerly held a place in the head.'

He now makes his careful and as usual beautifully illustrated study of the development of the Amphibian head and comes to this conclusion:

'Now, in the case of the fins of fishes, I have already shown that it is not possible to account for variation in position by the theory of inter- and excalation. Growth and transposition from one segment to another alone account for the facts. The same is probably true of the occipital condyle. There is not the slightest trace of the disappearance of segments behind

the vagus in the ontogeny of the Amphibia. We are familiar with the variation in the extent of the gill-region in Vertebrates by mere growth. Obviously the hind limit of the series of gill-slits varies backward or forward, according as certain segments cease to develop gills or take on the function of gill-formation. The posterior limit of the skull is doubtless altered in the same way, and the position of the occipital condyles may shift up or down the segmental series. There should, therefore, be no theoretical objection to accepting the anatomical and embryological evidence that the occipital region of the head in Amphibia contains only three segments. If segments could really disappear, leaving no trace behind, it would be hopeless to attempt to homologise segments in any two forms.'

This leads to his very important 1913 paper on 'Metameric Segmentation and Homology' where he develops this thesis to the full. As with fins, so with paired limbs. No one will deny that the fore limbs or hind limbs are homologous throughout the Tetrapods and that they can be traced back in an uninterrupted series to some common ancestral form; yet they are not necessarily made up of the same segments. The hind limb of the frog for instance occupies segments 8, 9, and 10, that of the salamander 16, 17, and 18, and that of *Necturus* segments 20, 21, and 22. These and many other facts are considered and lead him to give us this new conception of homology:

'In the Vertebrates, as in other animals, the organs and parts of two individuals are to be considered as homologous when they can be traced back to corresponding parts in a common ancestor, and not because they occur on the same segments. The homology is independent of the number and ordinal position of the segments which take a share in the formation of the organs. Any structure may apparently shift from one segment to another; and this is brought about neither by intercalation or excalation of segments, nor by redivision, nor by migration, but by a process of transposition. Organs may be homologous when they are composed of few or of many, of the same or of different segments, or are

not segmented at all. There are degrees of homology; it may be general or more special, complete or incomplete. The homology of two organs is complete when all their parts have been derived from corresponding parts in a common ancestor.'

When discussing vertebrate segmentation we should recall that he made an even more detailed study of this in the head of *Scyllium* in 1918.

In 1915 he published an account of a most delicate piece of work on the development of the chorda tympani (that twig of the hyomandibular branch of the facial nerve which supplies the organs of taste and salivary glands in the region of the lower jaw) in relation to the tympanic membrane and the structure of the middle ear in reptiles, birds and mammals. Its position in relation to the tympanic membrane had been a difficulty in accepting the conclusions of Reichert that the auditory ossicles of the mammal, stapes, incus and malleus, were derived from the columella, quadrate and articular respectively. If the tympanic membrane corresponded to the spiracle as had been thought, how is it that the chorda tympani passes above and in front of the former whereas it passes behind and under the spiracle? Goodrich clears up the whole matter in confirmation of Reichert's views. He shows that the tympanic membrane although now actually occupying the former position of the original spiracle, does not represent a covering of that opening; the tympanum develops as a separate diverticulum of the spiracular cleft (tympanic cavity) rather below and behind the spiracle proper which is more and more reduced as the diverticulum (tympanum) swells up to *take its place*. It swells up not only below and behind the spiracle but also below and behind the chorda tympani, so now this nerve passes above and in front of it. The difficulty is resolved. His paper is illustrated by plates of superb reconstructions of sections in which the different elements are shown in shadings of five different colours.

There are too many good things to look at all at once. In viewing any exhibition of works of art or science there comes a time when we must stop; there is a limit to what we can appreciate at one time, even if the works are familiar to us. We have

the catalogue; we know where they are to be found for future study. We must leave ourselves time, and here space, to consider our general impressions.

But there is still his greatest work of all, and we must hurry through the gallery of his vertebrate studies to reach it. As we pass we just note 'The Classification of the Reptilia', 1916 (also 1942), in which he points out the systematic value of the fifth metatarsal which is hooked in some and straight in others, and a kindred study of syndactyly as a key to the phylogeny of the Marsupials, 1935. We see his notes on the reptilian heart, 1919, and on the blastocoelic and enteric cavities in Amphibia, 1935.

His 'Proboscis Pores in Craniate Vertebrates', 1917, is a suggestion of the homology of the connections linking the coelom of the premandibular somites with the hypophysis, as found in the development of *Torpedo* and of the duck, with the opening of Hatschek's pit in *Amphioxus*, the proboscis pores of *Balanoglossus* and the water pores of Echinoderms, all to be regarded as coelomoducts. Here too are further studies on the Cyclostomes and fish: on the head of *Osteolepis*, 1919, the pectoral girdle of young Clupeids, 1922, the cranial roofing bones in the Dipnoi, 1925, the relationship of the Ostracoderms to the Cyclostomes, 1930, the spinal nerves of the Myxinoidea, 1937, and on the denticles in fossil Actinopterygii, 1942. We see his work on the vertebrates, as on the invertebrates, going on to the very end.

We now come to his *maximum opus*, that masterpiece: *Studies on the Structure and Development of Vertebrates*. It is all that his Cyclostomes and Fishes volume is, only more so: full of his own original work and his careful checking of the work of others. He refers to no fewer than 1,186 other works and it is illustrated by 754 text-figures of which again so many are his own. 'This work has been written', he says, 'in the hope that it may help advanced students and others engaged in teaching and research.' Every serious student of the vertebrates will acknowledge how fully his hope has been realised. His book is indispensable and it cannot be an extravagant prophecy to say that it will still be so in a hundred years' time. A senior zoologist in a recent letter

expresses the feelings of all: 'I never use his comparative morphology of the Vertebrates without an increasing admiration for the mind that conceived it or the hand that illustrated it.'

As with his *Cyclostomes and Fishes* volume it is so well known that any description of it in the space available would be superfluous. I prefer to use that space in quoting from his preface to show in his own words his grand conception; it will be useful too because so often the student dives at once into the text for the facts he wants and leaves the preface unread.

'It is not a complete treatise, but deals with certain subjects and problems of special interest and importance, some of which receive but scant notice in current text-books. My original intention was to cover the whole range of vertebrate morphology; but the preparation of this volume has taken so many years, that I thought it better to publish what is ready than to wait for the remainder which might possibly never be completed. The literature dealing with the Morphology of the Vertebrata is so vast, the accumulation of known facts so large, that students are apt to feel discouraged from the start, and to turn perhaps to some newer branch of zoological science. On the one hand, they may think that little remains to be done in so ancient a study; or, on the other hand, that its conclusions, for instance in Phylogeny, are so insecure that they afford little trustworthy evidence concerning the process of Evolution. It has, therefore, been my endeavour not only to give an account within reasonable compass of the facts already known and to discuss their significance, but also to point out where our knowledge is deficient; and where further research is desirable. During the last fifty years or so much has been accomplished, many old theories have been overthrown, some new conclusions have been firmly established; yet a great deal remains to be done, and new fields for research are continually being opened up.'

While not a complete treatise it deals very fully with the vertebral column, ribs and sternum, the median fins and paired limbs and limb girdles, the segmentation of the head, the skull, the skeletal visceral arches, the middle ear and ear ossicles, the

visceral clefts and gills, the vascular system and the heart, the air bladder and the lungs, the subdivisions of the coelom and the diaphragm, and the excretory and genital ducts. The nervous system and sense organs are less fully dealt with; but here he gives important new views on the evolution of the autonomic system. In the detail in which he planned it, no one person could have done more.

Some of the younger generation whose main interest is focused on the rapidly growing physiological, genetical or ecological branches of zoology may be inclined to think of Goodrich as an out-of-date morphologist—out of date because they regard morphology as a worked-out mine with little more to yield. His pupils never thought this. While Goodrich was largely engaged in morphological studies, he was never a morphologist pure and simple, in the sense of one who delights in unravelling and describing details of bodily structures as an end in itself. This end is of course important, but he was primarily a comparative anatomist and there is a big difference between being that and being a pure morphologist. The former compares animal structures with a view to discovering the course of evolution, tracing lines of phylogeny and building a classification as far as possible upon true relationship. Goodrich's passionate interest was not just in the details of the different kinds of nephridia and coelomoducts or scales and fins which he discovered; it was always centred upon the homologies they might reveal linking group with group: their evolutionary significance. Let me again quote from the preface of his great book:

'The triumph of the doctrine of Evolution has owed much in the past to the study of the structure and development of the Vertebrates, and the correct interpretation of their morphology still plays an important part in the elucidation of the evolutionary process. No other group of animals presents us with so complete a record of the divergent phylogenetic lines along which they have evolved.'

The elucidation of the evolutionary process—meaning the elucidation of the paths the process has taken rather than the causes underlying it—that was what he strove for all the time

and how remarkably successful was his quest. He had a genius for seeing the essentials and so selecting the profitable lines of attack. In that I think we see Goodrich the artist. Great landscapes are never photographic reproductions of nature; the artist emphasises those features which are essential to the beauty or character of his composition and lays less stress on others. Goodrich did not look at his animals with just the photographic eye of the pure morphologist. He saw much more; he had the insight enabling him to pick out from among the details those points which had significance for his picture; his evolutionary theme. Perhaps all great scientists are artists in their particular medium, but they become great only when, like Goodrich, they subject their artistic insight to the discipline and rules of the scientific method.

Goodrich never allowed himself to be carried away on wild flights of phylogenetic fancy; he was ever critical of those plausible speculations on descent which, often based only on flimsy similarities, bring discredit on the comparative method when they are held almost as creeds instead of being put forward as tentative hypotheses. He was always cautious. We have seen that in his 1907 paper on the phylogenetic position of *Polypterus* one can sense his leaning to the belief that it is a living Palaeoniscid; but he does not jump to this conclusion; he tests it step by step. In the paper of the following year on the scales of fish he writes: 'Not for a moment is it asserted that *Polypterus* is a living Palaeoniscid; but it is probably in the neighbourhood of this family that it will eventually find its place in the system of classification.' It was another twenty years before he allowed himself to write '*Polypterus* a Palaeoniscid?' and still with a question mark; it was another ten years before it seemed certain.

The discoveries Goodrich continued to make should show that comparative anatomy is not yet a worked out field. It is true that to-day it does not have the same attraction that it had; the new growing branches of biology are discovering more and more about the causes underlying the evolution, development, mechanism and behaviour of living things, so that it is natural that they should draw towards them those most curious about the nature of life. But the work of the great comparative anatomo-

mists like Goodrich is not obsolete and dead ; it has been providing a more and more reliable chart of the animal kingdom based on the course evolution has taken in the different phyla. It forms the essential background in the education of the zoologist, no matter in what particular field of research he intends to work. It makes sense of the diversity of form ; it shows what achievements the process of evolution is capable of.

While his research was mainly concerned with tracing the course of evolution it would be the greatest mistake to suppose that he was not interested in the causes underlying the process. In 1912 he wrote a little book in the People's Series called *The Evolution of Living Organisms* (second edition 1920) and in 1924 enlarged it considerably under the title of *Living Organisms: their Origin and Evolution*. No clearer accounts of our knowledge of the process of evolution had been written at the time these books were published and in spite of recent additions to the theory of the subject his *Living Organisms* is still one of the best introductions to evolution for the student. In the original 1912 edition see how clearly he emphasised the importance of both heredity and environment:

‘ An organism is moulded as the result of two sets of factors: the factors or stimuli which make up its environment, the conditions under which it grows up ; and the factors of inheritance, the germinal constitution, transmitted through its parent by means of the germcells. No single part or character is completely “acquired”, or due to inheritance alone. Every character is the product of these two sets of factors, and can only be reproduced when both are present. Only those characters reappear regularly in successive generations which depend for their development on stimuli always present in the normal environment. Others, depending on a new or occasional stimulus, do not reappear in the next generation unless the stimulus is present. In popular language the former are said to be inherited, and the latter are said not to be inherited. But both are equally due to factors of inheritance and to factors of environment ; in this respect the popular distinction between acquired and not acquired characters is illusory. In every



case it is the capacity to acquire, to become modified or to respond, which is really transmitted; the direction and extent of the modification depends on the stimulus. The presence of a given hereditary factor cannot be determined by mere inspection of the characters of an organism; the factor may be present, but the corresponding character fail to show itself owing to the absence of the necessary stimulus. On the other hand, dissimilar stimuli acting on different factors may give apparently similar results. Heredity must be defined afresh as the transmission of the factors of inheritance, and not as the reappearance of characters in successive generations.'

In 1921 he was President of Section D (Zoology) of the British Association at its meeting in Edinburgh and chose for the title of his presidential address: 'Some Problems in Evolution'. Here we find he had already appreciated that the genes form an interacting system subject to selection, so foreshadowing the conception of the gene-complex which has been so much developed in recent years, for he says:

'Thus natural selection preserves those factorial complexes which respond in a favourable manner. In other words an organism to survive in the struggle for existence must present that assemblage of factors of inheritance which under the existing environmental conditions will give rise to advantageous characters.'

During Goodrich's tenure of the Linacre Chair the Oxford department expanded both in space and scope. New laboratories for both undergraduate teaching and graduate research were added and, more important, the staff increased. Under his headship the Oxford school widened with the rapid development of zoology because he chose for his new colleagues young zoologists, all of whom were his own pupils, enthusiastic in different fields of work. Alongside his own researches were developing under his encouragement the embryological and evolutionary studies of G. R. de Beer, the work on breeding seasons and later on cytology and histochemistry of J. R. Baker, the ecological population studies of C. S. Elton, the ornithologi-

cal work of B. W. Tucker, the genetical and field evolutionary studies of E. B. Ford, the researches on nerve anatomy, physiology and regeneration of J. Z. Young and the work of the late J. A. Moy-Thomas on palaeozoic fish. More recently he had appointed H. K. Pusey, whose embryological evolutionary studies have been interrupted by war service, and P. B. Medawar who is subjecting the problems of 'growth and form' to mathematical treatment, and investigating the nature of the differences between individuals. In all this work, so much of it so different from his own, Goodrich took a great and sympathetic interest. While always ready to help with suggestions he never tried to divert the researches of his staff along lines other than of their own choosing.

He was from first to last a warm and valued friend of the marine biological stations at Naples and Plymouth; he worked repeatedly at each, and at home frequently obtained material from them.

In character Goodrich was quiet, reserved and unassuming; while those who did not know him well would have regarded him as somewhat shy and retiring, his close friends found in him a fund of amusing dry humour. In spite of his reserve he was a great teacher. We who have been his pupils will never forget his style in lecturing. As in writing, he had in speech that power of clear and logical presentation of complicated fact, and lucid explanation of theory. But the strength of his lectures was not in the spoken word; his speech was quiet, not forceful. The impression upon his students was made by a beautiful combination of verbal clarity with visual demonstration on the board. As he developed his exposition so also he developed drawings in coloured chalks; they were not just diagrams, they were not slow and laboured drawings, they were pictures, often optical sections in three-dimensional perspective, which grew before our eyes to build up the animal structures he was describing. Bones, blood vessels, nerves, were put in in just the right sequence to make understanding easy, put in, as he spoke, with the rapid sure touch of the artist. He made easy all those difficulties of visualising what is really happening in an animal's development when organs are being formed by the folding of

surfaces or the nipping off of this or that bud or diverticulum; with a growing series of sketches giving all the impression of solidity, we could not fail to follow the changing and subtle relationships of form he was describing. His drawings had life and reality in them; they made his lectures vivid and unforgettable. Story has it that on one occasion his Honours class asked permission for the rubbing out of his drawings to be delayed while they had them photographed; all enquiries I have made to try to obtain that photograph have failed. I should much like to see it reproduced and placed on record.

Another feature of his lectures should be specially mentioned because it was so much a part of his character: his extreme modesty. When he was describing his own contributions to zoology, and we have seen how many and great they were, he never gave the slightest hint to the student that they were indeed his own discoveries.

He took the keenest and kindest interest in the practical work of his class; he was always tolerant and sympathetic to the young student in difficulties and always ready to show him how to make closer observations and better drawings of what he saw. He encouraged his pupils to go out into the field; when the course on Protozoa came round he used to offer a book prize in the Honours class for the student who had collected and made drawings of the largest number of specimens; it was keenly competed for.

A great deal of his time must have been taken up by editing the Quarterly Journal; so many have testified to the unstinted help and advice which he gave towards the improvement of the papers they had sent him for publication.

Apart from his teaching and research he took an active part in the work of the University, serving on many committees. He was always much interested in the affairs of his College and for a time was Garden Master and Librarian. One of the senior Fellows in a letter writes of his life at Merton as follows: 'He never had an enemy and he is the only one I can remember who, in that somewhat strange life of a college, never lost his temper or fell out with anybody. He was the gentlest and kindest of men, but quite inflexible in following the course he felt right.'

His advice was of the greatest value in all artistic and architectural questions. He made a collection of photographs of all views of the precious parts of the college buildings, details of old and decaying stone carvings, woodwork and in fact anything that he considered would form a useful record to be of service to succeeding generations of students. He himself designed many new details, for example: common room chairs, fireplaces and brackets for lights in the quadrangle; it was he also who rescued from a lumber attic the remains of Wren's chancel screen of carved wood and had it adapted, according to his own designs, to beautify the reading rooms below the library.

It was in painting and travel that he sought relaxation from his scientific work. He travelled extensively and always returned home with a series of striking water-colours. He delighted in the play of strong sunlight and shadow on buildings, the more delicately graded light and shade on Alpine snows, or the subtle colourings in reflections. His clear colours and bold draughtsmanship captured the atmosphere of Venice, southern Italy, Greece, Tunis and Egypt, far away Java and Malaya, and many another scene of his many vacational wanderings. His sense of composition was just as keen. Occasionally he had exhibited in Bond Street, but it was only his intimate friends who realized the full scope of his power and versatility with the water-colour brush.

In this impression of his character and work I have reserved till the end that which is most personal: his very happy partnership with his wife, Dr. Helen Pixell Goodrich. Except in the two papers already referred to, in which they collaborated, they worked independently while sharing common scientific interests. They were devoted companions in their happy home and their wanderings together abroad. It is his work we are reviewing, not hers, yet no tribute to his life and achievement would be complete without a tribute to her as well. I am sure he would like it to be said, and I am sure it is the truth, that for his continued achievements in zoology up to the very end of his life we have in no small measure to be grateful to her. Those who him knew well from early years at Oxford, and with whom I have discussed his life, have each stressed the noticeable change

in health and happiness that came to him on marriage. Our deep sympathy goes out to her in her great personal loss and we admire her courage as she continues her work in his old department.

Edwin Goodrich has gone from us; his work and influence live on. All are agreed that he was the greatest comparative anatomist of his day; in the history of science his achievements will, I believe, rank as high as those of any predecessor in his field of work, surpassing even those of the great master who inspired him, Edwin Ray Lankester.

A. C. HARDY

## CHRONOLOGICAL LIST OF SCIENTIFIC WORKS PUBLISHED

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1. 'Note on a large Squid (*Ommastrephes pteropus*, Stp.)', *Journal of the Marine Biol. Association*, **2**, 314-21, 1892.
2. 'Note on a new *Oligochaete*', *Zool. Anz.*, **15**, 474-6, 1892.
3. 'On a new Organ in the *Lycoridae*, and on the *Nephridium* of *Nereis diversicolor*, O. F. Mull', *Q.J.M.S.*, **34**, 387-402, 1893.
4. 'On the Fossil *Mammalia* from the *Stonesfield Slate*', *Q.J.M.S.*, **35**, 407-32, 1894.
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7. 'On the *Coelom*, *Genital Ducts*, and *Nephridia*', *Q.J.M.S.*, **37**, 477-510, 1895.
8. 'Nephridia and Genital Ducts', *Zool. Anz.*, **19**, 494-5, 1896.
9. 'Notes on *Oligochaetes*, with the description of a new species', *Q.J.M.S.*, **39**, 51-69, 1896.
10. 'Report on a Collection of *Cephalopoda* from the *Calcutta Museum*', *Trans. Linn. Soc.*, **7**, 1-24, 1896.
11. 'On the *Nephridia* of the *Polychaeta*. Part 1. On *Hesione*, *Tyrrhena*, and *Nephthys*', *Q.J.M.S.*, **40**, 185-95, 1897.
12. 'Notes on the Anatomy of *Sternaspis*', *Q.J.M.S.*, **40**, 233-45, 1898.
13. 'On the Relation of the *Arthropod Head* to the *Annelid Prostomium*', *Q.J.M.S.*, **40**, 247-68, 1898.
14. 'On the *Reno-Pericardial Ducts* of *Patella*', *Q.J.M.S.*, **41**, 323-8, 1898.
15. 'On the *Nephridia* of the *Polychaeta*. Part II. On *Glycera* and *Goniada*', *Q.J.M.S.*, **41**, 439-57, 1898.

16. 'On the Communication between the Coelom and the Vascular System in the Leech, *Hirudo medicinalis*', Q.J.M.S., 42, 477-95, 1899.
17. 'Observations on *Syllis vivipara*, Krohn', Proc. Linn. Soc., 28, 105-8, 1900.
18. *The Holothuriodea* in the *Treatise on Zoology*, vol. iii, London, 1900.
19. 'On the Nephridia of the Polychaeta. Part III. The Phyllodocidae, Syllidae, Amphinomidae, etc., with Summary and Conclusions', Q.J.M.S., 43, 699-748, 1900.
20. 'On the Structure and Affinities of *Saccocirrus*', Q.J.M.S., 44, 413-28, 1901.
21. 'On the Pelvic Girdle and Fin of *Eusthenopteron*', Q.J.M.S., 45, 311-24, 1901.
22. 'On the Excretory Organs of *Amphioxus*', Proc. Royal Soc., Lond., 69, 350-1, 1901.
23. 'On the Structure of the Excretory Organs of *Amphioxus*'. Part I. Q.J.M.S., 45, 493-501, 1902.
24. 'On the Body Cavities and Nephridia of the *Actinotrocha* Larva', Q.J.M.S., 47, 103-21, 1903.
25. 'On the Dermal Fin Rays of Fishes—Living and Extinct', Q.J.M.S., 47, 465-522, 1903.
26. 'On the Branchial Vessels of *Sternaspis*', Q.J.M.S., 48, 1-10, 1904.
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**Studies on the Structure, Development,  
and Physiology of the Nephridia of Oligochaeta**  
**PART VIII: Biochemical Estimations of Nutritive  
and Excretory Substances in the Blood and Coelomic  
Fluid of the Earthworm and their Bearing  
on the Role of the Two Fluids in Metabolism**

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With 10 Tables

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## I. INTRODUCTION

IN Part VI of this series of memoirs (1) I recorded a number of experiments and observations on the excretory processes of the earthworm *Pheretima posthuma*. The presence of urea and ammonia in both the blood and coelomic fluid of the earthworm clearly indicated that both these fluids take their share in the transport of metabolic wastes from the tissues of the body to the nephridia. It was also found that the body-wall and intestine, the two main seats of metabolism in the

earthworm, were also the two main places of initial excretion of urea and ammonia; in fact, the proportion of urea and ammonia is higher in the body-wall and intestinal wall than in the coelomic fluid or blood or urine, the proportion of urea being the highest in the intestinal wall. No trace of uric acid was found in any part of the body. In order to determine the role of the nephridia in eliminating metabolic wastes from the blood and coelomic fluid, measurements were made of the osmotic pressure of the two fluids, and their protein and chloride contents were estimated. It was found that the osmotic pressure of blood was about 40 per cent. higher than that of coelomic fluid, that the protein content of blood-plasma was seven to eight times that of coelomic fluid-plasma, but that the chloride content of coelomic fluid was about 60 per cent. higher than that of blood. On the basis of these estimations and the corresponding ones on the urine of the earthworm, the conclusion reached was that the nephridia were concerned not only with the elimination of urea and ammonia, but that they also took part in the conservation of water, reabsorption of proteins and chlorides, and consequent regulation of osmotic pressure. It was further found that the nephridia also acted as 'storage kidneys' for the products of blood-destruction.

While working out the part played by the blood and coelomic fluid in the excretory processes of the earthworm, the problem presented itself as to why there should be two separate internal fluids in the body of the earthworm, while there is only one in the vertebrates.<sup>1</sup> What is the nature of the division of functions between the coelomic fluid and blood of an earthworm? The current view enunciated by Rogers (5) is that the blood of an earthworm is a solution of haemoglobin and serves as a carrier of oxygen, while the coelomic fluid is a carrier of food-materials to the various parts of the body and of metabolic wastes to the nephridia for elimination. This view was critically examined in respect of the transport of metabolic wastes and was found untenable, since it was proved that blood was as much concerned in the transport of urea and ammonia as the coelomic fluid. It

<sup>1</sup> Tissue fluid and lymph in vertebrates are not regarded as separate fluids, since lymph is derived from tissue fluid and the latter from blood.

has now been found that both blood and coelomic fluid contain creatinine as well in more or less the same proportions. The next question to examine is whether the coelomic fluid does actually carry food-materials to the various parts of the body, as is commonly believed. Michaelsen, as quoted by Winterstein (6, p. 551), also believed like Rogers that the coelomic fluid is a chyle-carrying system for the transport of food juices out of the gut into the blood. In vertebrates generally the blood by itself performs nutritive, respiratory, and excretory functions, besides a number of other subsidiary functions, although in lower vertebrates the coelomic fluid has an excretory function. Hawk and Bergeim (3) state with regard to the human blood, 'As (blood) plasma is the carrier of nutrition, we find it to contain glucose, amino-acids, fats, salts, &c. As carrier of excretory products it contains urea, uric acid, creatinine, acetone, carbon dioxide and many other substances in small amounts'. It was, therefore, considered advisable to make quantitative estimations of these substances in the blood and coelomic fluid of the earthworm, as they would provide a clue to the respective functions of these two internal fluids.

Since there are several methods for biochemical estimations of each of these different substances, I have, as far as possible, given full details of the particular methods employed by me so that my results can be easily checked by subsequent workers. The earthworm *Pheretima posthuma* was used throughout this work for the collection of blood and coelomic fluid, as this earthworm alone is available at Lucknow throughout the year.

I am very thankful to my former pupil and present colleague, Mr. L. N. Johri, for his great help in carrying out the various biochemical estimations recorded in this paper. I am also deeply indebted to my friends Dr. S. M. Sane of the Chemistry Department and Dr. M. S. Mangalik of the Pathology Department for their ready and ungrudging help and advice given to me at all stages of this work.

## 2. WATER CONTENT OF BLOOD AND COELOMIC FLUID

Before estimating substances of nutritive and excretory value in blood and coelomic fluid, it was thought best to estimate the

percentage weight of water in the two fluids. The results obtained are as follows:

TABLE I. *Water Content of Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (gm. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (gm. per 100 c.c.)</i>
1.	90.96	98.44
2.	87.72	98.65
3.	91.00	99.27
4.	90.47	98.47
5.	88.66	99.55
Average	89.76	98.87

Human blood-plasma contains 90 to 93 gm. of water per 100 c.c., and it is interesting to note that the blood of the earthworm also contains about 90 per cent. of water. The coelomic fluid-plasma, on the other hand, is much more watery as it contains about 99 per cent. of water. Whole coelomic fluid is less watery as it contains innumerable corpuscles floating in it.

Five c.c. of the blood or coelomic fluid-plasma was placed in a previously weighed crucible and weighed again to get the exact weight of the fluid. The fluid was now evaporated on a water bath. It was then dried at a temperature of 100° C. to 110° C. in an electric oven. The crucible was cooled and weighed, and again dried in the electric oven two or three times, cooled and weighed till the weight was constant. The difference between this weight and the original weight gave the weight of water lost in evaporation, from which the percentage weight of water was calculated.

### 3. ORGANIC NUTRITIVE SUBSTANCES IN BLOOD AND COELOMIC FLUID

The chief organic nutrient substances in the blood are glucose, amino-acids, and fats. As the methods of determination of these substances are standardized and their percentage value in human blood is accurately worked out, the same methods were employed in the determination of these substances in the blood and coelomic fluid of the earthworm.

## (a) Glucose.

Glucose was estimated first and the results are given in the following table:

TABLE II. *Glucose in Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
(Hagedorn and Jensen's titration method.)		
1.	135.40	Nil.
2.	116.82	"
3.	70.87	"
4.	125.67	"
5.	107.09	"
6.	118.59	"
7.	84.96	"
Average	108.50	"
(Folin and Wu's Colorimetric method.)		
1.	70.00	Nil.
2.	100.00	"
3.	85.00	"
4.	107.00	"
5.	96.80	"
Average	91.76	"

The most striking and important result obtained from these estimations is that the coelomic fluid contains no glucose whatever, while the earthworm's blood contains about the same percentage of glucose as human blood. According to Hawk and Bergeim (3), Hagedorn and Jensen's method gives the percentage of glucose in human blood at 75–105 mg. per 100 c.c.; in earthworm's blood this very method gave us 70 to 135 mg. of glucose per 100 c.c. Folin-Wu's method gave 70–107 mg. of glucose in earthworm's blood as against 90–120 mgm. in human blood.

As the two methods employed by me are standard methods and are adequately described in books on Biochemistry, they need not be described here. Hagedorn and Jensen's method is described in detail by Cole (2), while Folin-Wu's method is described by Hawk and Bergeim (3). It often happens that the protein-free filtrate of earthworm's blood after first filtration still shows a faint pinkish tinge; this is always removed either by

treatment with decolourizing charcoal or better by filtering the fluid twice or thrice, as otherwise it would lead to inaccuracy in the matching of colour in the colorimeter. Klett-Bio Colorimeter was used in all the colorimetric determinations.

(b) Amino-acids.

The results of estimations of amino-acid nitrogen are given in the following table:

TABLE III. *Amino-acid Nitrogen in Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
1.	6.00	Nil.
2.	6.10	"
3.	5.43	"
4.	7.10	"
5.	5.50	"
6.	6.10	"
Average	6.04	"

Human laked blood contains 5 to 8 mg. per cent. of amino-acid nitrogen, while the unlaked filtrate contains 2.3 to 3.7 mg. The blood of the earthworm having haemoglobin dissolved in the plasma is comparable with human laked blood and not with unlaked filtrate, and it is interesting to find that values of amino-acid nitrogen in earthworm's blood correspond closely to those of human laked blood. The next interesting point to be noted is that, like glucose, amino-acid nitrogen is also absent in the coelomic fluid.

Danielson's modification of the Folin method was employed for the determination of amino-acids. Two stock solutions were prepared, one containing 0.1 mg. of amino nitrogen per c.c. as glycine and the other 0.1 mg. amino nitrogen per c.c. as glutamic acid, each in 0.07 N HCl. plus 0.2 per cent. sodium benzoate. For making the standard, equal parts of these solutions were mixed and diluted with 0.07 N HCl. containing 0.2 per cent. sodium benzoate, giving it a strength of 0.05 mg. of amino nitrogen per c.c. To both the standard (1 c.c. standard and 9 c.c. water) as well as the tungstic acid filtrate of blood or

coelomic fluid (10 c.c. containing 1 c.c. of blood or coelomic fluid) were added 2 c.c. of 1.5 per cent. borax solution and 2 c.c. of freshly prepared 0.5 per cent. *b*-naphthaquinone sulphonic acid solution. Both tubes were kept in a dark closet for 24 hours. To each tube was added 2 c.c. of acid formaldehyde solution and 2 c.c. of 0.1 M sodium thiosulphate, and water added to make up to 25 c.c. After mixing for 4–5 minutes the two were compared in a Klett-Bio Colorimeter. The method and the calculation are described by Hawk and Bergeim (3, p. 432).

(c) Fats.

The results of the estimations of fats<sup>1</sup> are as follows:

TABLE IV. *Fats in Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
1.	185	Nil.
2.	215	„
3.	200	„
Average	200	„

The interesting result again is that although fats are present in the blood, they are absent in the coelomic fluid-plasma.

Fats were determined by the Soxhlet method using fat-free paper coils to absorb the liquids. 5 c.c. of each fluid was spread on a separate paper coil; the two coils together with a control coil were dried at a temperature below 100° C., and the fats were extracted from the paper coils with absolute ether in a Soxhlet apparatus as described by Hawk and Bergeim (3, pp. 206–7). The flasks containing fats were then heated at a temperature below 100° C. to a constant weight, using a safety water-bath. The weight of the fats in 5 c.c. of each fluid was thus obtained and the percentage value calculated.

#### 4. SALTS IN BLOOD AND COELOMIC FLUID

Of the salt contents, chlorides had already been estimated (1) and the results obtained showed that the coelomic fluid contained, on an average, about 80 mg. of chlorides per 100 c.c.,

<sup>1</sup> I am thankful to Mr. K. P. Srivastava of the Public Analyst's Department at Lucknow who kindly made these estimations for me.



while the blood-plasma contained about 50 mg., i.e., the coelomic fluid contained 60 per cent. more of chlorides than blood. Calcium, sodium, and inorganic phosphates were now estimated.

(a) Calcium.

The results of calcium estimations are as follows:

TABLE V. *Calcium in Blood and Coelomic Fluid*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
1.	16.00	21.20
2.	16.80	22.20
3.	17.60	23.20
4.	16.80	21.60
5.	18.00	22.80
6.	17.20	24.00
Average	17.06	22.50

In human blood calcium comes to 9–11.5 mg. per 100 c.c. and it is, therefore, interesting to note that the blood of the earthworm contains about 70 per cent. more of calcium per 100 c.c. than human blood does. Still more interesting is the fact that the coelomic fluid of the earthworm contains about 32 per cent. more of calcium than its blood does; in fact, the coelomic fluid of the earthworm contains twice as much of calcium as human blood does.

That the food of an earthworm, i.e., the earth, usually contains abundant calcium compounds is well known, but the fact that its blood and coelomic fluid contain so much of calcium leads to the obvious conclusion that the earthworm not only eats more calcium but that it is also able to absorb a much larger quantity of calcium than man does, since that is the only way by which it can maintain a high percentage of calcium in its blood and coelomic fluid. Evidently an earthworm requires a larger quantity of calcium for its metabolic needs than man does, but it seems that it absorbs very much larger quantities than it needs, and therefore it has a mechanism in its calciferous glands to excrete the excess of calcium in the form of calcium carbonate. An earthworm, therefore, does not suffer from calcium deficiency but from a surfeit of calcium. Robertson (4)

thinks that an earthworm has no control over its absorption of calcium and therefore has to excrete excess of calcium salts in the form of calcite concretions which escape into the oesophagus in a chemically inactive form. An earthworm has no exoskeleton and therefore the comparatively high calcium content of its coelomic fluid and blood can only be explained as a necessary condition for the ciliary activity of several structures in its body-cavity and the amoeboid movements of its blood- and coelomic corpuscles.

For the estimation of calcium, Kramer and Tisdall's method as described by Cole (2, p. 380) was followed. Calcium was precipitated as calcium oxalate, dissolved in sulphuric acid and titrated with 0.01 N. potassium permanganate. But as the earthworm's blood contains dissolved haemoglobin, iron was always removed first by evaporating the blood after addition of a little nitric acid, igniting the residue into ash, dissolving the ash in concentrated HCl and precipitating iron with ammonium hydrate.

(b) Sodium.

The results of estimations of sodium are as follows:

TABLE VI. *Sodium in Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
1.	75.35	159.28
2.	96.47	173.21
3.	99.88	193.90
4.	111.40	213.90
Average	95.77	185.07

Human blood-plasma contains normally 300 to 330 mg. of sodium per 100 c.c., while the chlorides are about 370 mg. per 100 c.c. In the earthworm the proportion of sodium in the coelomic fluid-plasma is about twice as much as in the blood. As the chlorides in the blood and coelomic plasma are only 50 and 80 mg. respectively (1), it is evident that while a small part of sodium occurs as sodium chloride, the greater part forms other combinations. It is well known that sodium chloride with

other salts helps to maintain the osmotic equilibrium in the tissues of the body.

Sodium was estimated by Weinbach's modification of Butler and Tuthill's method as described by Hawk and Bergeim (3, p. 472). After deproteinization with 20 per cent. trichloroacetic acid, sodium in the filtrate is precipitated in alcoholic medium as the triple salt, uranyl zinc sodium acetate, which is washed with acetone wash reagent (saturated solution of the triple salt in acetone) and titrated with sodium hydroxide, uranium, and zinc-forming amphoteric hydroxides. As complete details of procedure and calculation are clearly described by Hawk and Bergeim (3), they need not be repeated here. The only difficulty I experienced was that both in the case of blood and coelomic fluid a white precipitate appeared on the addition of acetone reagent. The entire precipitate did not completely dissolve in water and therefore the insoluble part was rejected on the supposition that the whole of the sodium salt must have dissolved and gone into the solute.

### (c) Inorganic Phosphates.

The results of the estimations of inorganic phosphates are as follows:

TABLE VII. *Inorganic Phosphates in Blood and Coelomic Fluid.*

<i>Serial No.</i>	<i>Blood-plasma (mg. per 100 c.c.)</i>	<i>Coelomic fluid-plasma (mg. per 100 c.c.)</i>
1.	17.85	1.85
2.	18.51	2.7
3.	17.16	2.7
4.	16.66	1.5
5.	17.09	1.3
Average	17.45	2.01

The normal inorganic phosphate content of human blood-plasma is about 3.7 mg. per 100 c.c. It is significant that the earthworm's blood contains eight to nine times as much of phosphates as human plasma does, while the proportion of phosphates in the coelomic fluid is very low.

The protein-free filtrate of blood and coelomic fluid was

obtained by treatment with 20 per cent. trichloroacetic acid, and was decolourized with phosphate-free charcoal. A standard solution of potassium phosphate containing 0.025 mg. phosphorus per 5 c.c. was prepared for the coelomic fluid and another standard containing 0.175 mg. of phosphorus per 5 c.c. was prepared for blood. Equal quantities of the filtrate and the standard phosphate were treated with ammonium molybdate and 1-amino-2-naphthol-4 sulphonic acid solution, and the colours matched in the colorimeter.

### 5. EXCRETORY SUBSTANCES IN BLOOD AND COELOMIC FLUID

Of the excretory substances, urea and ammonia had already been estimated (1), and therefore only creatinine has been estimated now in the two fluids. The results of the estimation of creatinine are as follows:

#### (a) Creatinine.

TABLE VIII. *Creatinine in Blood and Coelomic Fluid.*

Serial No.	Blood (mg. per 100 c.c.)	Coelomic fluid-plasma (mg. per 100 c.c.)
1.	3.75	2.67
2.	3.43	2.67
3.	3.43	2.67
4.	3.94	2.67
5.	3.22	2.70
Average	3.55	2.67

It is clear that, like urea and ammonia, creatinine is also present in both the fluids, the blood having a slightly higher proportion of it than the coelomic fluid.

In my previous paper (1) estimations of urea and ammonia only in urine were given; creatinine was not estimated; this deficiency has now been made good and the figures obtained are as follows:

TABLE IX. *Creatinine in the Urine of the Earthworm.*

1.	0.37 mg.	per 100 c.c.
2.	0.40 mg.	"
3.	0.65 mg.	"
4.	0.55 mg.	"
Average	0.49 mg.	"

Creatinine was estimated by Folin and Wu's method as described by Cole (2). Alkaline picrate is added to the protein-free filtrate and to a standard solution of creatinine zinc chloride and the two are compared in the colorimeter.

(b) Urea and Ammonia.

The proportions of urea and ammonia had already been determined (1) and are given below for comparison:

Urea.			
	<i>Blood</i> (mg. per 100 c.c.)	<i>Coelomic Fluid</i> (mg. per 100 c.c.)	<i>Urine</i> (mg. per 100 c.c.)
Average	3.18	2.52	3.24

Ammonia.			
	<i>Blood</i> (mg. per 100 c.c.)	<i>Coelomic Fluid</i> (mg. per 100 c.c.)	<i>Urine</i> (mg. per 100 c.c.)
Average	1.81	4.13	2.66

The conclusion already reached on the basis of the presence of urea and ammonia that both blood and coelomic fluid are carriers of excretory materials receives further confirmation now that the presence of creatinine has also been demonstrated in both blood and coelomic fluid.

## 6. DISCUSSION AND SUMMARY

In discussing the results recorded in the preceding sections of this paper, we may first consider the presence of the main organic nutritive substances in the blood and coelomic fluid of the earthworm. While the blood contains glucose, amino-acids, and fats in more or less the same proportions as in human blood, all these three substances are absent in the coelomic fluid-plasma. The obvious conclusion from this fact is that in the earthworm, as in man, blood-plasma is the carrier of nutriment to all parts of the body, and that coelomic fluid takes no part in the transport of nutritive substances. The commonly held view as enunciated by Rogers (5) and Michaelsen (6) that coelomic fluid is the carrier of nutriment to the various parts of the body is, therefore, clearly untenable. The coelomic fluid of the earthworm has no nutritive function at all.

As one would expect, calcium, sodium, and inorganic phosphates are present in both the fluids. Of these three salts, the proportion of phosphates is more than eight times as much in the blood as in the coelomic fluid. Phosphates are known to be necessary for carbohydrate and fat metabolism as also for the transport of fat; their presence in good proportion in the blood is, therefore, clearly associated with the nutritive function of the blood. Since the coelomic fluid has no nutritive function, its phosphate content is very low. As regards the proportion of calcium in the blood of the earthworm, it seems certainly high for a soft-bodied invertebrate without an exoskeleton, but it is even higher by about 32 per cent. in the coelomic fluid. Robertson (4) points out that calcium is essential for the normal activity of the neuro-muscular system, for amoeboid and ciliary movement, and for the clotting of the body fluids of most animals. It is also essential for all rhythmical movements and for keeping up the muscular tone. When we bear in mind the fact that the coelomic cavity of the earthworm contains a very large number of nephridia (innumerable in *Pheretima*) with ciliated funnels and extensive ciliated tracts within their bodies, the cilia of which keep up a regular rhythmical movement, the ciliated oviducal and spermiducal funnels, and the amoeboid corpuscles within the coelomic fluid itself, the importance of calcium in the coelomic fluid bathing all these structures becomes evident.

Sodium plays an important part in the acid base balance in the human blood and there is little doubt it plays the same role both in the blood and coelomic fluid of the earthworm, and thus helps to maintain osmotic equilibrium in the body of the earthworm. A part of sodium in both the fluids must be combined with chloride to form sodium chloride, but one cannot say at the present state of our knowledge how the rest of the sodium is combined.

The excretory function of the two fluids has already been demonstrated (1). My conclusions are further confirmed by the demonstration of the presence of creatinine in both these fluids as also in the urine.

The results of biochemical estimations of blood and coelomic

fluid of the earthworm and the conclusions derived from them may now be summarized as follows:

1. The proportions of different substances in the blood and coelomic fluid of the earthworm as compared with those in the human blood are given in the following table:

TABLE X. *Comparison of the Composition of Blood and Coelomic Fluid-plasma of the Earthworm, and the Human Blood-plasma.*

Serial No.	Constituent.	Concentration in blood (gm. per 100 c.c.)	Concentration in coelomic fluid-plasma (gm. per 100 c.c.)	Concentration <sup>1</sup> in human blood-plasma (gm. per 100 c.c.)
1.	Water	89.76	98.87	90-93
2.	Glucose	0.092-0.108	Nil	0.10
3.	Proteins	3.64	0.48	} 7-9
4.	Amino-acid N	0.006	Nil	
5.	Fats	0.20	Nil	
6.	Na	0.095	0.185	0.30
7.	Cl	0.05	0.08	0.37
8.	Ca	0.017	0.0225	0.01
9.	PO <sub>4</sub> (inorganic)	0.0174	0.0020	0.005
10.	Ammonia	0.004	0.0027	Nil
11.	Urea	0.0026	0.0025	0.02-0.04
12.	Creatinine	0.0035	0.0026	0.001

2. In the earthworm, blood alone is the carrier of nutritive substances to the various parts of the body, as is proved by the fact that it contains glucose, amino acids, and fats in more or less the same proportions as human blood does. The coelomic fluid, on the other hand, takes no part in the transport of nutritive substances, as is clear from the fact that it contains neither glucose, nor amino acids nor fats.

3. The coelomic fluid has higher calcium and sodium contents than blood, while the blood has a higher phosphate content. The higher calcium content of the coelomic fluid is probably associated with the rhythmical ciliary movements of several structures in the coelomic cavity, *e.g.*, nephridia, oviducal and spermiducal funnels, &c., while the higher phosphate content of the blood is associated with the part played by

<sup>1</sup> The figures in this column are taken from Samson Wright's "Applied Physiology" (1942).

it in carbohydrate and fat metabolism and the transport of fat.

4. Both the blood and coelomic fluid take part in the excretion of metabolic wastes. The presence of ammonia and urea had been previously demonstrated, and it has now been found that both fluids contain creatinine as well. Creatinine is also present in the urine of the earthworm along with urea and ammonia. The evidence is, therefore, absolutely clear that both coelomic fluid and blood take part in the excretory processes.

5. The coelomic fluid in its container, the coelomic cavity, must be looked upon as a 'private pond' of the earthworm which bathes all its structures and forms a suitable internal environment for the proper functioning of the different organs of the body. Its relatively higher water content probably serves to keep the skin moist even when the soil is relatively dry; it maintains contact with the external environment through the permeable body-wall, and by a proper balance of its calcium and sodium ions ensures the continued lashing of cilia of the various ciliated organs, the amoeboid movements of its corpuscles and the continued well-being of the animal as a whole. The fluid of the pond is constantly cleansed of its metabolic wastes by the elimination through the nephridia of urea, ammonia, and creatinine. The respective functions of the different corpuscles in the fluid have yet to be investigated.

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# The Effect of the Removal of the Lateral Appendix in the Embryonic Olfactory Organ of *Kaloula borealis* (Barbour)

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With six Text-figures

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## INTRODUCTION

THE lateral appendix is an ephemeral structure in the embryonic olfactory organ of the class Amphibia. Previous workers who investigated the nasal development of species of Amphibia also touched upon this structure and advanced opinions on its function (Hinsberg, 1901; Kawagae, 1933; Watanabe, 1936). The present writer made a complete and detailed study of the lateral appendix in *Rana nigromaculata* (Tsui, 1946). Tsui and Pan (1946) also described the development of this structure in *Kaloula borealis*. The present paper is an experimental study, the aim of which is to find out the significance of the existence of the lateral appendix in the larval life of *Kaloula borealis*.

## MATERIAL AND TECHNIQUE

Eggs and larvae of *Kaloula borealis* were collected from ponds and ditches in Kunming in the summers of 1942 and 1943. From the previous investigation (Tsui and Pan, 1946) it is known that when the larva reaches the length of 6-6.5 mm. the lateral appendix is being differentiated from the olfactory placode. Starting with larvae of this length, experimental material was sorted into the following seven groups:

First group—length of larvae, 6-8 mm.

Second group—length of larvae, 8.5-10.5 mm.

Third group—length of larvae, 10.5-12 mm.

Fourth group—length of larvae, 12-16 mm.

Fifth group—length of larvae, 16–20 mm.

Sixth group—length of larvae, 22–33 mm.

Seventh group—length of larvae, 35–40 mm.

The purpose of this grouping was to see the effect of removal of the lateral appendix at different stages of nasal development, if the removal would have any effect. Each group was again divided into two parts. In one division only one of the appendices was removed, while in the other both of them were removed. In the first group, about 500 individuals were operated on. In no group was the number of experimental larvae less than 150.

Removal of the lateral appendix was carried out under a dissecting microscope. To hold the larva on a glass slide for operation a thin pad of wet absorbent cotton was pressed down on the slide. The larva, previously measured, was placed on this pad back upwards. The cotton fibres made a rough and uneven surface, which kept the younger larvae steady enough for operation. For older larvae which possessed stronger musculature and could leap out of the pad, an additional wet cotton pad was used to cover the back and flanks of the larva, leaving its head and tail free. The edges of this pad were pressed down on the lower pad with a pair of forceps. Thus tucked up, the tadpole was unable to squirm. The lateral appendix was sucked off by means of a micro-pipette connected with a piece of rubber tubing, the free end of which was held in the operator's mouth. In the earlier stages—the second, third, fourth, and fifth groups—the larvae are quite transparent. Their olfactory placode including the lateral appendix contains much more pigment than the skin and connective tissue. It is easy to find the appendix and to remove it. In the later stages, when the cartilaginous nasal capsule has formed, it is hard to see it under the dissecting microscope. It could be only approximately located by judging its anatomical position at each stage. Difficulty was also experienced in the material of the first group. The appendix at this time has not fully differentiated out of the olfactory placode as a separate structure. The supporting tissue, too, is not yet well developed and the primitive skin contains a good deal of pig-

ment and some yolk-granules. All this makes the animal opaque and its tissues disintegrate easily during the operation. Instead of the micro-pipette, a glass micro-needle had to be used to remove the anlage of the appendix in the first group. Therefore, the operation on the first and the last three groups was not always successful. In some cases the appendix was not, or not completely, removed; while in others a part of the olfactory placode was excised together with it.

After the operation the larvae were kept in 8-inch earthen vessels in the laboratory. Mortality was high (about 50 per cent.) but the number of experimental animals was large enough to take care of that. Reared in these small vessels in the laboratory, the growth of the larvae was retarded in comparison with those living in their natural environment. When operated upon at the earlier stage, including the first five groups (their length, up to 20 mm.), and transferred to small vessels in the laboratory, the larvae grew up to the length of 25–30 mm. At that point their development was arrested. When operation was made at later stages, viz. the last two groups, their growth went a little bit farther. Then it was arrested, too. None of them passed through metamorphosis. The same result obtained in the controls which accompanied the experimental animals of the first, third, and last groups. The inhibition of development was probably due to the unsuitable environment in the laboratory.

Experimental animals of the first three groups were fixed at the following intervals: 2, 5, 8, 12, 19, 26, 33, and 47 days after the operation, the older groups were fixed at longer intervals. In fixation, Spuler's fluid was used. Following the ordinary procedure, material was embedded in paraffin. Serial sections were cut  $8\mu$  in thickness and were stained with haematoxylin and eosin. For the second to fifth groups inclusive, four to five specimens from each batch of fixed material were prepared for study. For the other groups, in which difficulty in operation had been experienced, more specimens were cut so as to secure a similar number of animals on which the operation had been successful. Serial sections of normal specimens prepared for the previous paper (Tsui and Pan, 1946) were used as controls.

## OBSERVATIONS

First Group.—In most cases no change in the structure of the olfactory organ has been found, whether one or both lateral appendices were removed. Three different stages are taken to illustrate the structure of the olfactory organ after the removal of the lateral appendix. For the sake of convenient comparison, specimens with only one lateral appendix excised are used in the illustrations, so that the nasal organ operated upon can be compared with the intact one of the same specimen.

The control larva of 7.5 mm. in length shows that the external naris was closed up and the opening of the middle lumen was on its way to extend anteriorly. The experimental larva of the same length fixed two days after operation shows the same condition on both sides of the nasal cavity (Text-fig. 1 A). In a later stage (larva length, 12 mm.) the control larva shows the formation of the anterior lower sac, the closing up of the distal part of the entrance canal and that the opening of the middle lumen was extending anteriorly. In the experimental larva of the same length, five days after operation, the same situation obtained (Text-fig. 1 B). Besides, the form of the whole organ developed normally. Text-fig. 1 C represents the middle region where the lateral appendix is connected with the placode. The form of the nasal organ with excised appendix is identical with that of the intact organ. Still later, when the larva reached the length of 24 mm. three important developmental changes normally took place: first, the formation of dorsal evagination, second, the appearance of the medial trough indicating the beginning of separation of the upper and the posterior lower sac and, third, the formation of the medial nasal gland. The experimental larva when grown to the same length shows exactly these changes (Text-fig. 2 A and B).

In about 30 per cent. of the sectioned material, however, alterations in the olfactory organ are found after operation. Three types of modifications have been noted.

In the first type the change was slight. A small part of the nasal epithelium with which the appendix had been connected was removed with it, so that this section of the epithelium

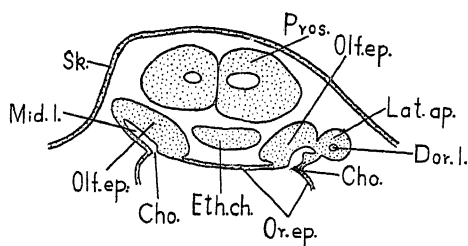


FIG. 1 A

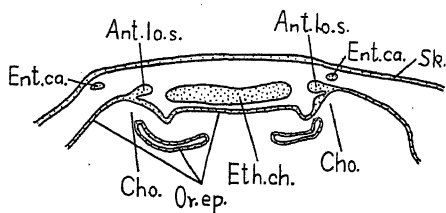


FIG. 1 B

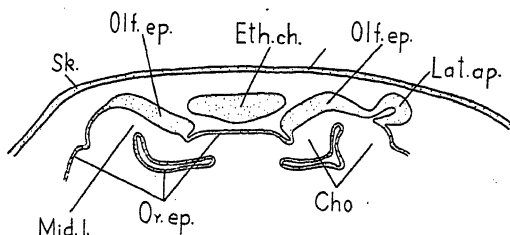


FIG. 1 C

## TEXT-FIG. 1.

Cross-sections through the olfactory organ, from which the right lateral appendix was removed.  $\times 50$ . A. Through the lateral appendix (length of larva, 7.5 mm.); the section was slightly oblique so that the right olfactory placode appears much longer than the left. B. Through the anterior-most part (length of larva, 12 mm.). C. Through the lateral appendix of the same larva as B. *Antlos*, anterior lower sac; *Cho*, choana; *Dorl*, dorsal lumen; *Entca*, closed entrance canal; *Ethch*, ethmoidal part of chondrocranium; *Latap*, lateral appendix; *Midl*, middle lumen; *Olfep*, olfactory epithelium; *Orep*, oral epithelium; *Pros*, prosencephalon; *Sk*, skin.

appeared shorter than that on the opposite and intact side (Text-fig. 3). Another effect was that in closing the wound after

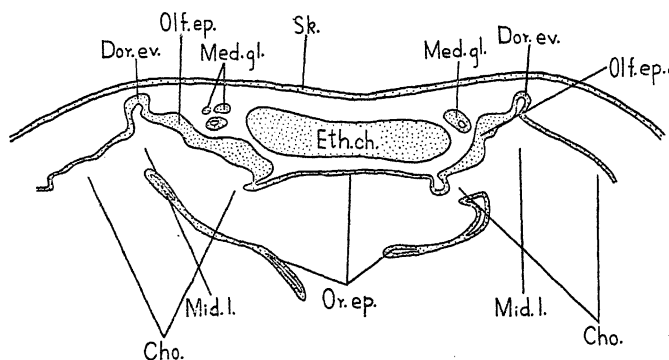


FIG. 2A

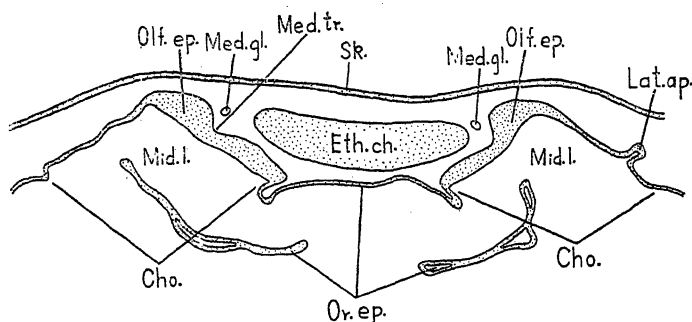


FIG. 2B

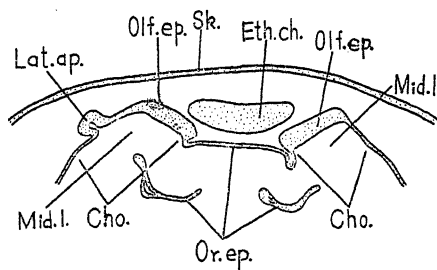
TEXT-FIG. 2.

Cross-sections through the olfactory organ, from which the right lateral appendix was removed (length of larva, 24 mm.).  $\times 50$ . A. Through the dorsal evagination. B. Through the lateral appendix. *Dorev*, dorsal evagination; *Medtr*, medial trough; *Medgl*, medial nasal gland; other abbreviations as in Text-fig. 1.

the excision, growth was more active resulting in greater thickness of the epithelium both at the wounded spot and posterior to it.

In the second type the modification was more profound. The olfactory epithelium was laterally compressed. It bent down-

ward and inward. In addition to this lateral compression, the nasal organ was compressed also antero-posteriorly. This is shown clearly by examining serial cross-sections. The anterior-most parts of both nasal organs (one intact, the other operated upon) begin to appear at the same level, but the organ operated upon ends much sooner and is found to be one third shorter. The distortion is particularly marked at the ventral part. That portion of the oral epithelium, which forms the floor of the posterior prolongation of the nasal organ, is, because of the



TEXT-FIG. 3.

Cross-section through the lateral appendix of the olfactory organ, from which the left lateral appendix was removed (length of larva, 12 mm.).  $\times 50$ . Abbreviations as in Text-fig. 1.

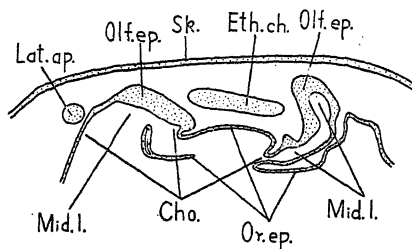
distortion, thrust forward so that a cross-section at the level of the appendix cuts through the whole length of the floor, whereas in the intact organ on the other side only a very small portion of the floor is cut through at this level. As a consequence of the lateral compression and the shifting forward of the floor of the posterior prolongation, the middle lumen became a narrow irregular cavity and the normally wide open choana, a narrow slit (Text-fig. 4).

In the third type there was no obvious modification in the form of the olfactory organ, but a large portion of the nasal epithelium had been torn off with the appendix and only a part remained (Text-fig. 5).

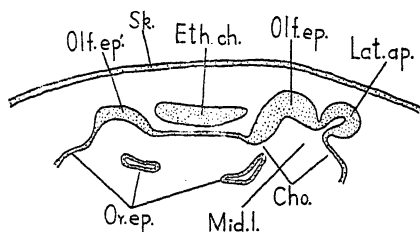
All cases in which the above modifications have been observed are found in earlier fixed specimens, viz., those fixed within 8 days of the operation. In specimens fixed later in life such



changes were very rare, and among those fixed 47 days after operation, no deformation is found. It is most probable that the changes were the temporary responses to the stimulation of injury. The animals recovered later on.



TEXT-FIG. 4.



TEXT-FIG. 5.

Fig. 4. Cross-section through the lateral appendix of the olfactory organ, from which the left lateral appendix was removed (length of larva, 12 mm.).  $\times 50$ . Abbreviations as in Text-fig. 1.

Fig. 5. Cross-section through the lateral appendix of the olfactory organ, from which the right lateral appendix was removed (length of larva, 11 mm.).  $\times 50$ . *Olf.ep.*, remaining portion of the olfactory epithelium; other abbreviations as in Text-fig. 1.

As stated above, the olfactory organ underwent post-operational changes only in 30 per cent. of the specimens, and as these changes were again largely restricted to the earlier fixed larvae, it is obvious that these changes did not arise as a consequence of removal of the lateral appendix *per se*, but were due to the injury incidental to the operation, because the olfactory placode at this early stage was very soft and the lateral appendix was not yet fully differentiated out of it. To remove the lateral appendix only a micro-needle could be used. In such an opera-

tion it was easy to cause injury and the consequent deformation in the contiguous epithelium and adjoining parts as was the case in the second type of alteration. If the injury was slight, only the contiguous epithelium was affected as found in the first type. If it was severe, the whole or the major part of the olfactory placode was damaged and only a part of the epithelium was left behind as happened in the third type. That the changes were merely due to the incidental damage in the operation was also borne out by the fact that when a faulty operation left the appendices intact but evidently injured the placode, changes also took place.

**Second Group.**—As in the foregoing group, in most of the specimens their nasal organ after operation was normal in its development (cf. Text-figs. 1 and 2). In some of the earlier fixed larvae a slight change was observable at the place of excision. The epithelium in section appears shorter and thicker at the wound spot and posterior to it. This modification is similar to that described as the first type in the foregoing group. In a few specimens among the earlier fixed batch, there was found a small protrusion at the very place where the lateral appendix was sucked off (Text-fig. 6 A). That portion of the olfactory epithelium below the appendix suffered distortion, so that just posterior to the appendix it formed a loop enclosing a small lumen (Text-fig. 6 B). As in the second type of modification found in the first group, here also we find a shortening of the nasal organ and a shifting forward of the floor of its posterior prolongation so that the latter presents its whole length in the section illustrated in Text-fig. 6 A, while on the intact side its whole length appears a number of sections later (Text-fig. 6 C). As a result of the alterations in the olfactory and oral epithelium there were a narrowing down and distortion of the middle lumen (Text-fig. 6 A and B). This change was quite similar to that obtaining in the second type described in a preceding paragraph, but here the deformation was less in extent. The olfactory placode had become larger at this stage, and its epithelium was well developed and therefore more resistant to injury. The appendix was also well delimited and could be neatly sucked off with a micro-pipette. But injury was not always avoidable. The

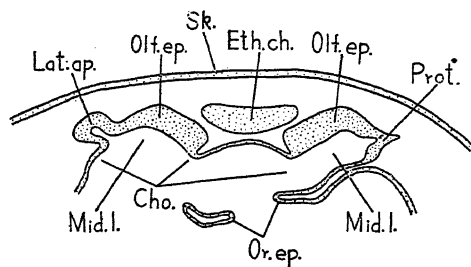


FIG. 6A

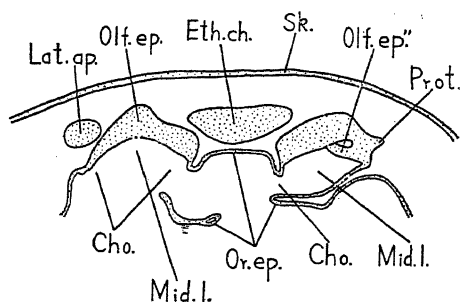


FIG. 6B

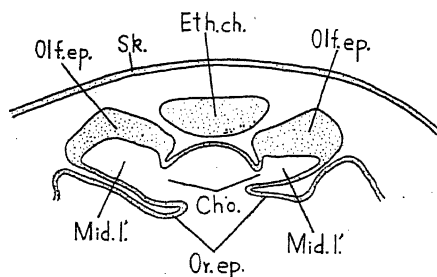


FIG. 6C

## TEXT-FIG. 6.

Cross-sections through the olfactory organ, from which the left lateral appendix was removed (length of larva, 11 mm.).  $\times 50$ . A. Through the lateral appendix. B.  $20\mu$  posterior to A. C. Through the posterior prolongation. *Midl'*, posterior extension of the middle lumen; *Olfep''*, portion of the olfactory epithelium, which bends over toward the placode; *Prot*, protrusion; other abbreviations as in Text-fig. 1.

distortions of the olfactory epithelium and of the floor of the posterior prolongation were nothing but a result of disturbance during the operation.

No further modification could be observed in this group.

**Third Group.**—In the earlier fixed larvae there were a few which showed a slight modification similar to the first type of change described under the first group. The nasal organ of all others developed normally in the absence of the lateral appendix.

The removal of the lateral appendix in the last four groups caused no post-operational change in the nasal development. In the experimental animals of all these groups the nasal organ developed normally irrespective of the age at which they were fixed.

It is clear that when the nasal organ is in a more advanced stage, its resistance to injury becomes increasingly great. No change took place, though the disturbance during operation was still liable to occur.

As stated above, larvae operated upon in their earlier developmental stages never grew more than 30 mm. in length, and even those operated upon later did not pass through metamorphosis (which occurs when the larva reaches 48 mm. in length) under the conditions in which they grew. It is impossible to say whether any change in the nasal development would take place in experimental animals during late larval life and during and after metamorphosis. The form and structure of the nasal organ of the oldest animals in all of the seven groups were similar to those of the controls at the same stage. This fact supports the view that in the absence of the lateral appendix the further development of the nasal organ is in no way different.

#### CONCLUSION

The excision of one or both lateral appendices in no way affected the course of development of the olfactory organ. This experiment does not show that this structure has any role to play. One might regard it as a vestigial organ, having lost the function that it once had. Such a phylogenetic interpretation is, however, rendered difficult by the fact that though the lateral appendix is present in all *Amphibia* investigated, it has, as far

as the writer knows, never been found in fishes. Intensive researches on this structure in the more primitive Amphibia may possibly give some clue to its function. At present we can only conclude that the function of the lateral appendix remains entirely unknown, if it has or had any function at all.

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# The Effect of Removal of the Anterior Lower Sac on the Lateral Appendix in the Embryonic Olfactory Organ of *Kaloula borealis* (Barbour)

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With Plates 8 and 9

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## INTRODUCTION

In previous investigations on the development of the olfactory organ of *Rana nigromaculata* (Tsui, 1946a) and *Kaloula borealis* (Tsui and Pan, 1946) it was found that the lateral appendix is suddenly arrested in its development at the appearance of the anterior lower sac and gradually degenerates as this sac further grows. It was suggested (Tsui, 1946b) that the lateral appendix may be the forerunner of the vomero-nasal organ in the early life of the Amphibia and is functionally soon replaced by the latter organ. The removal of the lateral appendix was found, however, to have no effect—at least in the larval stages—on the development of the nasal organ of *Kaloula borealis* (Tsui, 1946c). On the other hand, it is possible that removal of the anterior lower sac out of which the vomero-nasal organ is differentiated would have effect on the activity of the lateral appendix and cause it to persist. In order to put this idea to test the following investigation has been carried out.

## MATERIAL AND TECHNIQUE

From the previous investigation on *Kaloula borealis* (Tsui and Pan, 1946) it is known that when the larva reaches the length of 10 mm., the anterior lower sac begins to differentiate from the medio-anterior region of the olfactory placode. Larvae from 11 mm. to 38 mm. in length were collected from ponds and ditches in Kunming in the summers of 1942 and 1943. This material was sorted into the following seven groups:

- First group—length of larvae, 11 mm.
- Second group—length of larvae, 14 mm.
- Third group—length of larvae, 18–20 mm.
- Fourth group—length of larvae, 23–27 mm.
- Fifth group—length of larvae, 28–32 mm.
- Sixth group—length of larvae, 34 mm.
- Seventh group—length of larvae, 36–38 mm.

The purpose of this grouping was to see the effect, if any, of removal of the anterior lower sac at different stages of nasal development on the fate of lateral appendix. Each group was again divided into two parts. In one division only one of the anterior lower sacs was removed, while in the other both of them were excised.

The general procedure of operation used in the previous investigation (Tsui, 1946) was followed. A micro-pipette was used to suck off the anterior lower sac. In the larvae of later stages (last five groups) difficulty was experienced in locating exactly the anterior lower sac, since the cartilaginous nasal capsule had formed and made the sac not clearly visible under the dissecting microscope. It could be located only approximately by judging its anatomical position at each stage. Therefore, the operation was not always successful. To take care of this, more specimens of the older material were operated upon to insure an adequate number of individuals from which the anterior lower sac was successfully removed.

The rearing of the animal and histological procedure were the same as followed in the previous investigation. The growth of larvae in the laboratory was identical with that obtaining in the previous experiment in which the lateral appendix was excised (Tsui, 1946). Experimental and control larvae within 20 mm. in length grew to the length of 25–30 mm. Larger larvae brought to the laboratory grew a little bit further. None of them passed through metamorphosis.

#### OBSERVATIONS

In the experimental larvae of the first four groups the removal of the anterior lower sac produced detrimental effect on the

lateral appendix. From measurements of its length and width the appendix was found to be always shorter and narrower in comparison with that of the control animal. Observations on its detailed structure show that this was due to the decreasing in number of its epithelial cells. Counts were made of the nuclei of the cells in a section through the middle region of the lateral appendix. The cells themselves in the basal layers of the appendix are so crowded that their boundaries are hardly distinguishable. Therefore, the number of nuclei was taken to represent the number of cells in the appendix. From a large number of counts it was found that the appendix of the nasal organ from which the anterior lower sac was excised contains 20 per cent. less cells than that in the normal appendix.

This difference is, however, not restricted to the lateral appendix but extends to the whole olfactory placode. As the appendix, the placode is also shorter and narrower in the absence of the anterior lower sac. Its olfactory epithelium is thinner and consists of cells that look less healthy by reason of their thinner contents. The histological structure of the placode is similar to that of the lateral appendix, but the epithelium is more stratified and the cells are still more crowded. The number of its cell layers can be ascertained by counting the layers of nuclei. In the absence of the anterior lower sac the placode in a given cross-sectioned area contains one or two layers of cells less than the normal placode of a corresponding area. The effect on appendix and the placode is easily demonstrable when only one anterior lower sac was excised; for here the difference in the size of the appendices and the placodes on either side can be seen at a glance (figs. 1, 2, Pl. 8). In this connexion, it may be pointed out that this effect manifested itself soon after the removal of the anterior lower sac and persisted as long as the larvae lived under the laboratory conditions. For instance the olfactory organ illustrated in fig. 1 was from a specimen fixed five days after the operation, while that illustrated in fig. 2 was fixed forty-seven days afterwards. Both showed the same effect of the removal of the anterior lower sac.

In a few cases when both of the anterior lower sacs were removed the operation was not equally successful; one of these



two sacs was completely excised while a part of the other was left behind. In such cases the appendix and the olfactory placode on the side of the incompletely excised sac were also reduced in size but to a less extent than those on the opposite side. This condition is demonstrated in fig. 3, Pl. 9.

As pointed out in the opening paragraph, the appearance of the anterior lower sac synchronizes with the stoppage in growth of the lateral appendix and the latter slowly degenerates as the sac grows. The results described above show that the removal of the anterior lower sac from the younger larvae appears to hasten the degeneration of the appendix. With its removal the appendix is always shorter and narrower as compared with control animals. The growth of the placode of the experimental animal lags also behind that of the normal ones.

This deleterious influence on the appendix and the placode produced by the removal of the anterior lower sac, however, was restricted to younger larvae—those of the first four groups. In the last three groups the excision of the sac had no effect whatever on the olfactory placode. Its size and finer structure are similar to those of the normal structure. But on the lateral appendix the removal of the sac from the older animals had a beneficial effect. In its absence the degenerative process of the appendix was slowed down. This structure is larger and its cells are richer in contents when compared with control specimens (fig. 4, Pl. 9). This result was always obtained whether one or both of the anterior lower sacs were excised. In cases when one of the two sacs was incompletely excised, the appendix on that side was smaller than on the opposite side where the excision of the sac was complete but was still larger than in the control specimen of the corresponding developmental stage.

As stated above the younger larvae (first three groups) did not grow beyond the length of 30 mm. under the laboratory conditions. Within this span of growth developmental changes in the placode which obtain in normal larvae also occurred in the experimental animals. Thus the normal course of the development of the placode was not affected by the removal of the anterior lower sac, though in the younger groups this removal affected the growth of the placode somewhat adversely.

## DISCUSSION

As had been expected, the removal of the anterior lower sac had the effect of retarding the degeneration of the lateral appendix. But, surprisingly enough, this effect was obtained only in the older larvae. For larvae within 27 mm. in length the excision of the sac had the opposite effect of furthering the degenerative process. This early effect is hard to explain. It was certainly not due to physical injury caused by the operation. The anterior lower sac is situated far to the front of the appendix. Its removal in no way caused physical disturbance of the latter structure.

It is unfortunate that larvae reared in the laboratory made only limited growth. They did not pass through metamorphosis. What effect the absence of the anterior lower sac would have on the various parts of the nasal organ in its later developmental stages could not be ascertained from the material available.

The olfactory placode was adversely influenced by the absence of the anterior lower sac in the younger larvae only. Up to the time of the arrest in growth of the experimental larvae in the laboratory, the normal course of development of this principal olfactory structure was not affected by the removal of the anterior lower sac. Presumably its further development would continue to be normal in the absence of the sac.

It would be very desirable to know the final fate of the lateral appendix in the absence of the sac. Would the harmful influence of the removal of the sac in younger larvae on the one hand, and its beneficial influence in the older ones on the other, continue to be registered by the appendix in its later life?

The anterior lower sac is the embryonic structure which later gives rise to vomero-nasal organ or recessus medialis and its lateral extension, the cavum inferius, in the adult olfactory organ. When this sac is removed from the larva the adult organ will probably be devoid of these derivative sacs. If this is the case, it may throw some light on the function of the vomero-nasal organ concerning the function of which we have thus far only conjectures.

In order to be able to answer the questions raised above it is planned to repeat the present experiment and to rear experimental animals in as natural conditions as possible in hopes of getting them to pass through metamorphosis.

### SUMMARY

The removal of the anterior lower sac from the embryonic olfactory organ of *Kaloula borealis* produced two different and opposite effects on the lateral appendix. In larvae 11–27 mm. in length the excision of the sac had a harmful effect and hastened the degeneration of the appendix. When the same operation was made on the larvae 28–38 mm. in length, however, the degeneration of the appendix was retarded. Early removal of the sac had also deleterious effect on the olfactory placode but did not, within the experimental period, influence its normal course of development.

Since the experimental animals made only limited growth in the laboratory, the later effects of the removal of the anterior lower sac remain unknown. It is planned to rear experimental larvae under more natural conditions.

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— 1946b.—“Morphological observations on the fate of the lateral appendix in the embryonic olfactory organ of *Rana nigromaculata* Hallowell.” *Ibid.*, 87.  
— 1946.—“The effect of removal of the lateral appendix in the embryonic olfactory organ of *Kaloula borealis* (Barbour).” *Ibid.*, 87.  
— and Pan, T. H., 1946.—“The development of the olfactory organ of *Kaloula borealis* (Barbour) as compared with that of *Rana nigromaculata* Hallowell.” *Ibid.*, 87.

### EXPLANATION OF PLATES

All figures drawn with the aid of a camera lucida.

#### PLATE 8.

Fig. 1. Cross-section through the olfactory organ, showing the reduction in size of the right lateral appendix and the olfactory placode after the removal of the right anterior lower sac. Length of larva at the time of operation, 12 mm., and of fixation, 14 mm.  $\times 110$ .

Fig. 1 A. Camera lucida outline of cross-section through the anterior-most part of olfactory organ of the same specimen as fig. 1 to show the excision of the right anterior lower sac.  $\times 50$ .

Fig. 2. Cross-section through the olfactory organ, showing the reduction in size of the left lateral appendix and olfactory placode after the removal of the left anterior lower sac. Length of larva at the time of operation, 14 mm., and of fixation, 30 mm.  $\times 75$ .

Fig. 2 A. Camera lucida outline of cross-section through the anterior-most part of olfactory organ of the same specimen as fig. 2 to show the excision of the left anterior lower sac.  $\times 30$ .

#### PLATE 9

Fig. 3. Cross-section through the olfactory organ from which the left anterior lower sac was completely and the right one incompletely removed. The section shows less reduction in size of the lateral appendix and the olfactory placode on the right side. Length of larva at the time of operation, 12 mm., and of fixation, 14 mm.  $\times 110$ .

Fig. 3 A. Camera lucida outline of cross-section through the anterior-most part of olfactory organ of the same specimen as fig. 3 to show the complete excision of the left and the incomplete excision of the right sac (*Antlos*).  $\times 50$ .

Fig. 4. Cross-section through the olfactory organ, showing the retardation in degenerative process of the right lateral appendix after the removal of the anterior lower sac on that side. The olfactory epithelium in the median part of the right placode was incidentally sucked off on the removal of the sac, causing the placode to appear narrower than the left one. Length of larva at the time of operation, 28 mm., and of fixation, 30 mm. The difference in the thickness between the placodes shown in figs. 2 and 4 was merely due to individual variation.  $\times 75$ .

Fig. 4 A. Camera lucida outline of cross-section through the anterior-most part of olfactory organ of the same specimen as fig. 4 to show the excision of the right anterior lower sac.  $\times 30$ .

#### EXPLANATION OF LETTERING

*Antlos*, anterior lower sac; *Cho*, choana; *Entca*, closed entrance canal; *Ethch*, ethmoidal part of chondrocranium; *Latap*, lateral appendix; *Medgl*, medial nasal gland; *Olfpl*, olfactory placode; *Orep*, oral epithelium; *Sk*, skin.



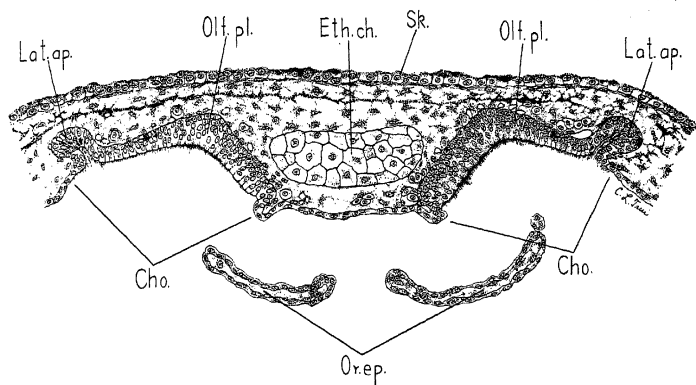


FIG. 1

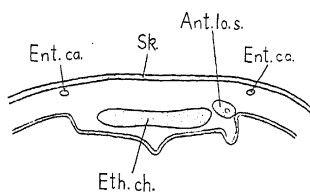


FIG. 1a

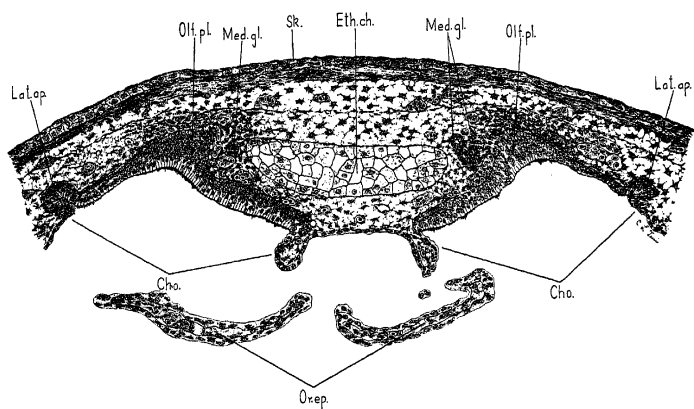
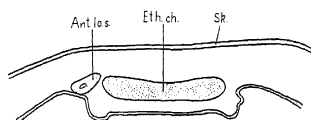


FIG. 2





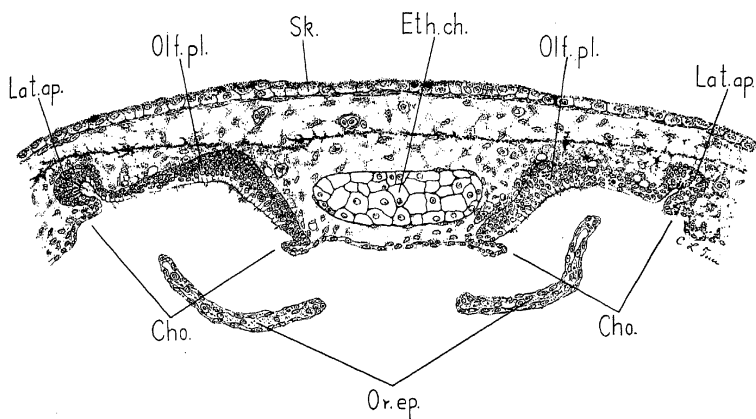


FIG. 3

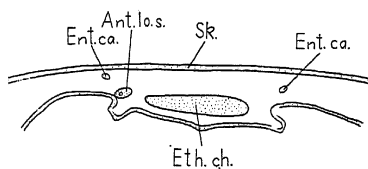


FIG. 3a

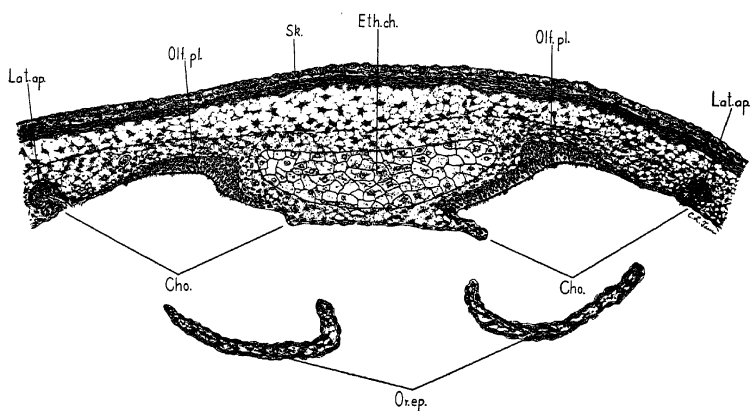


FIG. 4

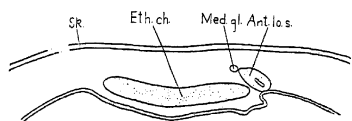


FIG. 4a





# The Formation and Structure of the Chorion of the Egg in an Hemipteran, *Rhodnius prolixus*

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With 13 Text-figures

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## INTRODUCTION

THE problem of the structure and permeability of insect eggshells is of considerable importance in agriculture and applied biology. However, very little work appears to have been carried out in this field, especially in relating the passage of water and materials designed to kill the egg to the morphology and biochemistry of the shell substance. The form of the egg has attracted the attention of systematists (as well as many of the earlier microscopists), and its external characteristics have found an important place in the identification of species. Unfortunately, on account of early work, many names and definitions applied to the layers and regions of the shell have accumulated. There are many ill-defined terms and a rather speculative literature on the site, nature, and importance of the micropylar and other specialized areas of the shell; this has led to much confusion.

The recent works of Slifer (1937, 1938), Slifer and King (1934), Jahn (1935 *a*, 1935 *b*, 1936), and Cole and Jahn (1937), carried out on the egg-membranes of grasshoppers, have described a large portion of the structure and permeability of this egg-shell. The Orthoptera, however, have an unusual method of waterproofing their cuticles, using an oily substance instead of the more usual wax (Ramsay, 1935; Wigglesworth, 1945; Beament, 1945 *a*, 1946 *d*), and in some cases this may be spread over the eggs or oötheca. These cannot, therefore, be considered as reasonably characteristic eggs.

The observation of Ongaro (1933) is of great importance. He found that a waxy material, containing approximately 80 per cent. paraffins, and making up 5.7 per cent. of the shell

weight could be removed from the vacated egg-shells of *Bombyx mori* by extracting them with a lipoid solvent. This at once presents the possibility that the waterproofing mechanism of typical egg-shells may be similar to that of most insects' cuticles, i.e. a very thin wax layer (Wigglesworth, 1945; Beament, 1945).

On the physiological side there have been good recent accounts of the relation of survival and water-loss of eggs to temperature and humidity (Johnson, 1934, 1940; Clarke, 1935). Among these, Evans (1934) has shown that in eggs of the sheep blowfly, *Lucilia sericata*, the shell is responsible for most of the waterproofing of the egg, but that some impermeability is retained when the shell is removed, providing the vitelline membrane is left intact. No attempt has been made, however, to correlate the chemical nature and structure of each layer or region of an egg-shell with its effect on the permeation of water and other materials through the shell.

In the present paper, the formation, structure, and permeability of the unspecialized part of the chorion and of the cap will be described. The structure of specialized areas, with special reference to the micropyles (Beament, 1946 *b*) and the waterproofing mechanism (Beament, 1946 *c*) will be discussed elsewhere.

#### MATERIAL

The egg of the reduviid bug, *Rhodnius prolixus* Stål, was chosen for this study. The culture was standardized as follows:

*Rhodnius* adults were kept in jars in an incubator, and laid eggs, usually at night, on strips of blotting-paper. Eggs were removed each morning, and placed in the same incubator in 3 in. by 1 in. tubes, the open tops of the tubes being covered by coarse gauze. The culture was maintained at 25° C. and 70 per cent. relative humidity.

#### METHODS

Most methods are explained in the text, but the initial problem of investigating the structure, staining reactions, and chemistry of the layers of the shell, necessitated some method of observing sections of the shell. The shells of insect eggs are

usually too brittle to obtain more than fragmentary sections by the standard methods of embedding and sectioning. The more complex regions of the shell are usually smashed. Until a sufficient knowledge of the chemistry of the shell had been obtained, it was impossible to devise a method of softening the shell sufficiently, while still leaving it in a normal morphological state, and for most of the work the following simple and rapid method was used:

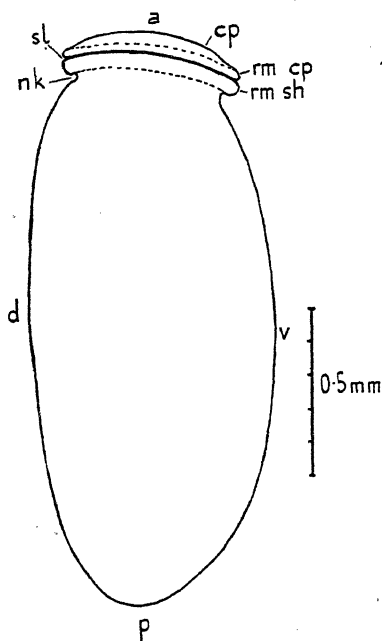
A small drop of glycerine jelly, diluted with twice its volume of water, was placed near the edge of a watch-glass. The egg to be sectioned was orientated in this drop, so that its long axis lay along a radius of the glass with the cap facing the centre. Ethyl chloride was sprayed on to the drop until it was frozen, and the egg was cut into two symmetrical halves by a stroke of a very sharp scalpel. These fragments will be referred to below as 'half-shells'. The halves were rapidly transferred to Ringer's solution and the contents washed out. After staining, &c., the half-shell was mounted in a cavity slide with the flat cut edge in contact with No. 0 coverslip, and the round side against the bottom of the cavity. In this way the surface of the cut edge could be observed in optical section under the 1/12 in. oil-immersion objective of the microscope.

From the properties of the shell layers outlined below, a procedure has been obtained for softening the shell sufficiently to cut sections at  $8\mu$  in the ordinary way. This has been used particularly to elucidate the micropylar region and other specialized areas (and see Beament, 1946 *b*).

### Shell Terminology.

The egg of *Rhodnius* (see Text-fig. 1) is an ovoid body approximately 2.5 mm. long and 0.7 mm. at its maximum diameter. At the anterior end the shell is constricted to about 0.5 mm., the neck, for a distance of about 0.1 mm., and this expands into the rim of the shell which, with its overhanging lip, is the thickest part of the shell. The rim of the cap is sealed on to this rim by a complex junction, the seal. The cap is a shallow convex disc, of circular outline, placed asymmetrically on the end of the shell.

The egg has a uniform pink colour when laid, but this is due entirely to pigmentation of the yolk, and, at a later stage of development, to the pink colour of the embryo (Wigglesworth, 1942). The shell itself is white and translucent, though the



TEXT-FIG. 1.

The egg of *Rhodnius prolixus*. *a*, anterior; *cp*, cap; *d*, dorsal side; *nk*, neck; *p*, posterior; *rmcp*, rim of cap; *rmsh*, rim of shell; *sl*, seal; *v*, ventral side.

vacated shell may occasionally show interference colours. The shell is sufficiently transparent for grosser details of the developing embryo, such as the eyespots, to be visible through it. The cap, however, is pale amber in colour, and is more opaque.

The whole of the external surface of the egg-shell is sculptured, being divided into irregular hexagons (Text-fig. 2), each with a pit at the centre, the follicular pit. There are also pits over the surface of the cap, but differing in structure from those of the main shell.

### The Layers of the Shell.

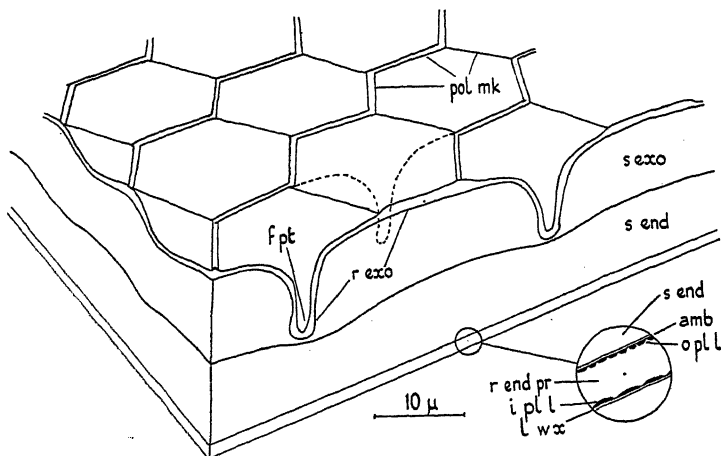
The term chorion has usually been applied to the whole or part of the shell of insect eggs. Snodgrass (1935) defines the chorion as that part of the shell which is secreted in the follicle of the ovary. But, one of the layers on the inside of the completed shell may be produced before or after the egg is released from the follicle (Beament, 1946 c). It is, therefore, proposed to amend the definition of the chorion to 'that part of the egg lying outside the oocyte cell membrane, which is secreted by the follicle'. Layers added by the oocyte, zygote, or embryo will not be included.

The terminology used hereunder attempts to divide the many layers of the shell according to their method of formation, chemical nature and function, while at the same time conserving a normal convention of nomenclature. Galliard (1935) divides the *Rhodnius* egg-shell into an outer layer, which he calls the 'chorion', and an 'inner membrane'. There is no doubt, however, that he has confused the inner portion of the true chorion with the layers added during embryonic development. As early as 1887 Korschelt had stated that the chorion was a double layer, and most subsequent authors have made a primary division of the egg-shell into an exochorion and an endochorion. Among these can be quoted Johnson (1934) on the eggs of the *Rhododendron* bug, Slifer and King (1934), and Christophers (1945) on the eggs of *Culex*. The layers of the *Rhodnius* egg-shell will therefore be subdivided within such a primary division.

Text-fig. 2 shows a diagrammatic section through the unspecialized part of the shell of the *Rhodnius* egg. As will be shown later, the outer or exochorion layers are secreted by the follicle in their second discrete secretory phase; they provide the mechanical rigidity of the shell, and their substance is not very permeable to water. They contain the whole of the follicular pits and the sculptured part of the shell. They are composed throughout of chemically similar material, secreted in that form by the follicle cells, and are subdivided on physico-chemical properties into two layers. There is, thus, a thin outer resistant layer, which does not vary greatly in thickness

over the whole shell and a soft layer forming the greater part of the exochorion, but subjected to great variations in thickness and accounting for the sculptured appearance of the shell.

The inner group of layers secreted by the follicle make up the endochorion and comprise the soft flexible part of the shell.



TEXT-FIG. 2.

Diagrammatic representation of a fragment of the completed shell.

Note the polygonal markings of the shell surface (*polmk*), each with a follicular pit at its centre (*fpt*). The resistant exochorion layer is thickened at the base of each pit and at the surface ridges (*rexo*). *sexo*, soft exochorion layer; *send*, soft endochorion layer. Inset: The detailed structure of the resistant endochorion layers. *amb*, amber layer; *ipll*, inner polyphenol layer; *opll*, outer polyphenol layer; *rendpr*, resistant endochorion protein layer; *lwx*, primary wax layer.

They are subjected to great variations in thickness in various specialized areas of the shell, but there are only superficial indications of the polygonal follicular pattern. The layers are formed in the first phase of secretory activity, and are all composed of a proteinaceous material, modified by further secretions from the cells; the subdivisions are based on these modifications.

The outermost endochorion layer is the soft endochorion, a thick layer over the unspecialized shell, but reduced to an extremely thin lamina in the neck and cap. It consists of pro-

tein material with only slight modification. Beneath this, the resistant endochorion is the most highly organized region of the shell. This layer is thin, and extends over the whole inner shell surface with very small variations in thickness. The central lamina is composed of material very similar to the soft endochorion, though more resistant to chemical attack. It is bounded on either side by two series of irregular islands of material containing polyphenols, the inner and outer polyphenol layers. Lying between the outer polyphenol layer and the soft endochorion there is a lamina which is atypical, for it is the only layer of the shell with a marked colour; all the other layers are translucent and colourless. This component forms a thick layer over the cap, but in the main part of the shell it is so thin that its presence can be detected only by its effect on the penetration of large molecules through the shell. Because of its colour, this layer will be referred to as the amber layer of the endochorion.

Neither the exo- nor the endo-chorion gives the shell its great resistance to the passage of water. The water-impermeable layer is a product of the oocyte (and therefore not part of the chorion), which lines the inner polyphenol layer. This is the excessively thin primary wax layer (Beament, 1946 *a*, 1946 *c*).

#### THE DEVELOPMENT OF THE EGG-SHELL IN THE OVARY

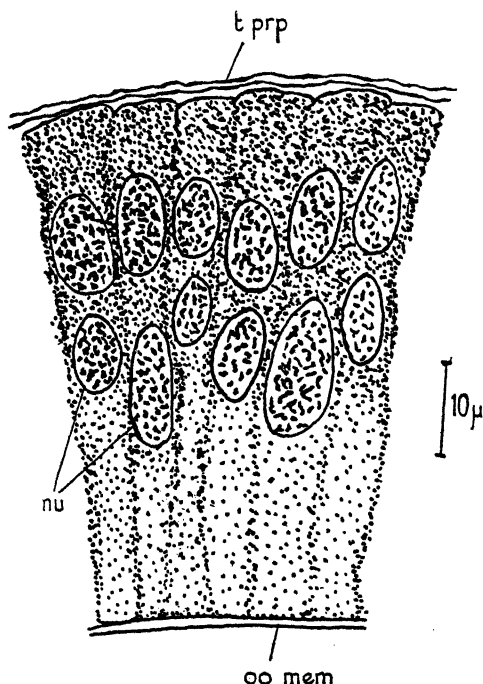
Each ovary of *Rhodnius* consists of seven telotrophic ovarioles. In each ovariole, cells are proliferated from a terminal filament and differentiated into oocytes, nurse-cells, and those which will form the follicle. In the vitellarium the egg-cells accumulate yolk, and when the oocyte has attained its full size the process of chorion formation starts. The whole ovariole is enclosed in a structureless membrane, or tunica propria.

Eggs with complete chorions are released from their follicles into a reservoir consisting of the pedicels of the ovarioles, together with the calyx and lateral oviducts. A sphincter muscle at the base of the lateral oviducts withholds these eggs until they traverse the vagina for fertilization and laying.



### The Follicle.

During the second ovarian stage, while the nurse-cells retain contact with the oocyte, the follicular cells are multiplying and taking up their position around the egg-cell. When nutrition

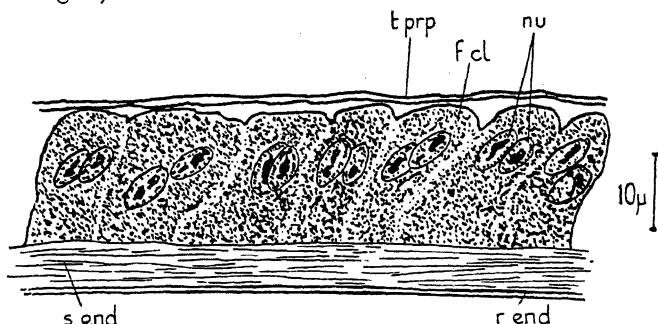


TEXT-FIG. 3.

Section through the follicular cells surrounding an oocyte about 0.4 mm. diameter. Note the elongate cells, each with two nuclei (*nu*). *oome*, oocyte membrane; *tprp*, tunica propria.

is taken over by the follicle, its cells have been arranged as a single layer round the oocyte (and see Musgrave, 1937, &c.). In this final preparatory stage the follicle cells undergo amitosis and become binucleate. This process has attracted much attention (Gross, 1901; Köhler, 1907; Murray, 1926). At this stage the follicle is roughly spherical with a diameter of approximately 0.4 mm. Its tightly packed cells are elongate (in

length some  $60\mu$ ), while the large ovoid nuclei are almost the same width as the cells containing them; the nuclei thus lie one above the other along the length of the cell. (This may account for descriptions by some authors of two rows of follicle cells.) The cell cytoplasm nearer the surface of the oocyte is clear and does not stain readily, while that distal to the egg-cell stains more strongly and is granular. The nuclei are packed with granular material, which may indicate that they are active in the process of secreting yolk material for the egg-cell (Text-fig. 3).

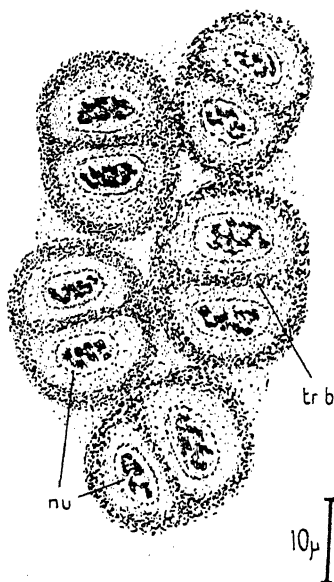


TEXT-FIG. 4.

Longitudinal section through follicle cells (*fcl*) during the secretion of the unspecialized soft endochorion. The cytoplasm is granular, and both cytoplasm and nuclei (*nu*) stain deeply. *rend*, resistant endochorion; *s end*, soft endochorion; *tprp*, tunica propria.

As the oocyte increases in size it becomes elongate, due in part to the pressure of the tunica propria, for if it is released from this enfolding membrane it reverts to a spherical shape. The increase in surface area allows the follicle cells to flatten until they form a layer  $15-20\mu$  thick; they do not undergo further division. Thus, when the oocyte has reached its final size, the paired nuclei of each follicle cell can lie side by side, and are so placed during the remaining life of the follicle (Text-fig. 4). The cells are apparently identical over all regions of the oocyte, though they are slightly longer over the anterior end (nearer the germarium), where the cap will eventually be formed. In surface view (Text-fig. 5) the follicle cells are roughly hexagonal. Each nucleus is surrounded by a clear region, while

the cytoplasm of the cell is made up of more dense material. The outer dense cytoplasm extends down between the twin nuclei, giving the appearance of a double cell. However, there is no trace of a cell membrane between the two nuclei. Apparently the direction of this transecting bar bears no obvious relation to the axes of the egg. It will be seen later that, in contrast, the boundary of the whole binary cell is imprinted



TEXT-FIG. 5.

Surface view of follicle cells during the secretion of the soft endo-chorion layer. *nu*, nuclei; *tr b*, transecting bar.

on the outer layers of the shell, whereas the transecting bar is but vaguely represented in the sculpture.

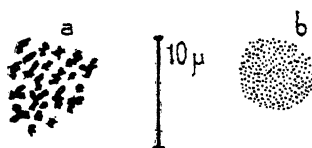
It is at this stage, when the oocyte has reached its full size, that the process of shell formation starts. The egg-cell membrane which, up till now, has been transporting material from the follicle cells to the yolk-cavity, becomes the vitelline membrane, and is the base or substrate for the deposition of the chorion. At this point, movements take place in the cells

of the anterior end which determine those areas where the neck, rims, and cap will be formed (and see Beament, 1946 *b*).

### RESISTANT ENDOCHORION

#### Inner Polyphenol Layer.

The first indication of shell formation is the deposition of a series of irregular droplets or islands of material over the whole surface of the vitelline membrane. These stain very deeply with ammoniacal silver nitrate solution, giving the typical pink-brown colour of the polyphenol reaction (Lison, 1936). The droplets average  $2\mu$  in diameter, and are shown in



TEXT-FIG. 6.

Appearance of the inner polyphenol layer (*a*) and outer polyphenol layer (*b*) after staining with ammoniacal silver nitrate.

Text-fig. 6. They remain as islands throughout the whole life of the egg (p. 433), and later form the substrate for the primary wax layer (Beament, 1946 *a* and *c*). The material of which they are composed is extremely resistant to the action of strong mineral acids and alkalis; it is of the same order of resistance as the amber layer (see p. 429). When pieces of the vacated shell are heated in strong nitric acid saturated with potassium chlorate (hereinafter referred to as chlorated nitric acid), all layers of the shell dissolve except for the resistant endochorion with polyphenol droplets attached, protected by the amber layer on the outer surface and the primary wax layer on the inside. Finally, this dissolves in the extremely strong oxidizing agent, leaving a number of granules which appear to be the polyphenol droplets.

It is, of course, incorrect to describe these islands as being composed of polyphenol only; they must consist of small quantities of protein, very heavily tanned, and condensed with polyphenol elements. Polyphenols are themselves evanescent

substances, leached out by water, removed by fixatives and certainly broken down by mineral acids unless they are thoroughly attached to proteins. There is a polyphenol reaction in the thicker layers of the endochorion before they have been treated with water, but after soaking in water for twenty-four hours, or boiling in water, only the islands give this indication with ammoniacal silver nitrate. Similarly, the granules are the only part of the shell to stain in cold Millon's reagent (primarily an indicator of benzene ring compounds); other regions of the shell require heat to stain. It is not definitely established whether the follicle or the oocyte, or a combination of the activity of both is responsible for the secretion of this layer, since it is almost impossible to obtain evidence for the presence of polyphenol in fresh tissue by using the silver nitrate method. Staining fresh follicular material in ammoniacal silver nitrate at this stage reveals masses of droplets concentrated round the peripheral regions of the base of each bicell. These might be polyphenols, but many substances which form part of the normal content of cells will mask the true reaction. After fixation, polyphenols are washed out of tissues (and see Wigglesworth, 1946). On the other hand, the unfixed vitelline membrane does not give a polyphenol reaction at all strongly. Since, also, only the follicle can have formed the outer polyphenol layer (p. 407), it is most likely that it will have formed this layer, too. It can, therefore, be included quite justifiably under the term 'chorion' (and see also p. 433).

The granules in the shell are quite invisible until after staining. Köhler (1907) describes a series of granules, differentiated by haematoxylin, on the inner surface of the egg-shell of *Nepa cinerea*, and Slifer and King (1934) have stated that the endochorion of the grasshopper egg is granular. These may be homologous structures.

### Protein Layer.

As soon as the inner polyphenol layer has been formed, the follicle starts to secrete the resistant endochorion protein layer. At this stage, and during secretion of the subsequent endochorion layers, the follicle cells stain deeply throughout their

cytoplasm with basic stains, indicating that they are packed with protein material; their reactions to stains are identical to those of the layer they are secreting (see below). This is the first secretory phase of the follicle. The nuclei contain one or more large central bodies, together with a number of peripheral granules (Text-fig. 4). A uniform layer, approximately 1 to  $1.5\mu$  thick is secreted over the egg. It does not bear any impression of the polygonal cells which are producing it, in contrast to the layers added later, and has no visible laminations (cf. soft endochorion). This layer has the following properties:

It is readily penetrated by water and water-soluble stains of comparatively large molecular size, and stains very strongly in picric acid, iodine, borax carmine, basic fuchsin, &c., though less strongly in acid fuchsin. When placed in strong nitric acid for a few moments and transferred to 0.88 ammonia solution, a bright orange colour is produced (xanthoproteic reaction), while, if heated to boiling in a drop of 0.2 per cent. triketohydrindine-hydrate in 50 per cent. glycerine, the layer becomes a bright blue colour (ninhydrine reaction). All these reactions indicate that protein is the main constituent present. Warm Millon's reagent in 50 per cent. glycerine turns the layer pink (contrast with the polyphenol droplets which stain in the cold).

When the egg-shell consists of this layer only, there is but the faintest staining reaction with ammoniacal silver nitrate, showing an almost complete absence of polyphenols, but after the outer polyphenol layer has been secreted, this protein layer stains very deeply with the typical pink-brown colour which is not diminished by soaking in water for a short time, though it is removed by boiling pieces of shell in water. The polyphenol content of this layer must, therefore, be added during the process of formation of the outer polyphenol layer.

As stated above, when the completed egg-shell is broken down in cold 'chlorated' nitric acid, the resistant endochorion is the only layer which remains. Similarly, it is the last layer to be dissolved in fused potash. However, until secretion of the exochorion is in progress, it is readily dissolved by strong

nitric acid and cold concentrated aqueous potash. This at first gives the impression that the protein layer under consideration becomes progressively more resistant with time, and that tanning, polymerization, and similar processes are proceeding slowly while the rest of the shell is being formed. This is not quite correct. In the complete and waterproof shell, the resistant endochorion is bounded on the outside by the amber layer (see below) and on the inside by the primary wax layer (Beament 1946 *a* and *c*). Both these substances are remarkably resistant to attacks by cold acids and alkalis; the wax layer allows their penetration only when the temperature of the attacking solution is above the melting-point of the wax. Hence the resistant endochorion protein is protected by these inner and outer layers, and immersion of the whole shell in solvents does not give a true index of the solubility of the protein layers.

When the inner protective layer (wax) is removed by boiling vacated shells in chloroform, cold concentrated potash, injected into the interior of the shell, attacks the protein layer very slowly (more slowly than when it is first formed and when wax has not been secreted over it). Similarly, strong nitric acid attacks the protein layer more rapidly when it is first formed than after the whole shell has been completed. Some form of 'hardening' process must, therefore, have taken place.

Although all the staining properties of this component and the histology of the cells which secrete it indicate that protein forms its main constituent, the isolated layer is apparently unaffected by trypsin or pepsin at any pH. When it is eventually broken down in potash or chlorated nitric acid, no oil is released (see exochorion and amber layers).

It would seem, therefore, that the protein becomes slightly more resistant by some forms of tanning and polymerization processes which proceed as the shell ages. Verson (1884), Tichomiroff (1885), and Farkas (1903) have shown that egg-shell material contains a high percentage of sulphur. Tichomiroff puts the sulphur content of 'chorionin' in the shell of *Bombyx mori* as 3.67 per cent. by weight, and Verson gives the figure of 4.38 per cent. The term 'chorionin' as used by these authors apparently includes all layers of the shell. If

this shell contains similar material to the *Rhodnius* egg-shell, then the amount of sulphur present cannot be explained by the protein, lipid, or any other component identified above or below. It can therefore be suggested that this sulphur may be taking part in linking together the protein components; such a process could be referred to as vulcanization. It seems possible that sulphur used in this way would account for the resistance of the shell to chemical solution.

### Outer Polyphenol Layer.

The follicle cells now secrete a second layer of islands of material containing large quantities of polyphenols, over the whole surface of the protein layer. The chemical properties of these droplets are identical with those of the inner polyphenol layer (see above), but the islands are much smaller, for they are of the order of  $0.2\mu$  (Text-fig. 6). At the same time the polyphenol material is added to the protein layer (see above). Köhler (1907) figures a series of droplets in the central 'endochorion' region of the shell of *Nepa cinerea*, but in this case, he states that they are much larger than those on the inside of the shell. There is, of course, no evidence that they are 'polyphenol droplets'.

### Amber Layer.

The outer polyphenol layer acts as a substrate for the deposition of the 'amber' layer. This is an excessively thin lamina over the unspecialized area of the shell, and cannot be detected microscopically from sections of the chorion. The following experiments show that such a layer must be present:

When eggs in the early stages of shell-formation are removed from the ovary, enclosed in their follicles, and fixed for three days in aqueous Bouin's solution, the yolk hardens so that the follicle can be readily stripped away and the oocyte is left enclosed in the incomplete endochorion. However, it is found that the surface of the shell is extremely hydrophobe. The yolk is heavier than water, and so the fixed oocyte can be immersed for three days, at the end of which it will still float on water because of the low contact angle. This at once excludes the



hydrophobe property from being due to the tanned nature of the polyphenol surface.

The surface of the layer is readily wet by 50 per cent. alcohol (in which the oocyte sinks), and also by chloroform and other lipophilic substances, but it is not made hydrophilic by boiling in chloroform, benzene, or other wax solvents. The egg is not waterproof at this stage and dries up completely in the course of two hours in a desiccator at room temperature. Though it has been shown (Beament, 1946 *b*) that the micropylar holes penetrate the layer, such perforation will not account for so rapid a rate of water-loss. This layer cannot, therefore, contribute towards waterproofing the egg. Further evidence for this is shown by the penetration of stains through the layer.

### Experimental.

Incomplete, non-waterproof eggs were placed in Ringer's solution, and their caps removed. The contents were washed out with a fine pipette, taking great care not to rupture the shell. In order to support the delicate membrane, the inside of the shell was plugged with a wad of cotton-wool, or short ends of very thin-walled glass tubing (drawn to a diameter slightly less than that of the egg) inserted while still immersed in the liquid. The specimens were transferred to distilled water to wash out the salt (from the Ringer), and dried in a desiccator. Stains were then introduced into the rear end of the shell membrane through a very finely drawn pipette. In order to control injection the pipette was connected with a mercury manometer made of rubber pressure tubing with one limb supported by a fine worm screw. Suppression of this limb caused stain to enter the cavity of the egg-shell, and the amount injected could be controlled to within less than a 1/50th cub. mm. Such control was very necessary, since unless it was certain that no stain penetrated the shell from the outside, or crept round the cut edge, the experiment would have been invalid. In these experiments the cavity was only half-filled with stain, the shell being supported in a trough formed by sealing two glass plates on to a third with their plane edges opposed in a very narrow 'V'. The pipette was mounted on an adjustable screw, so that it

could be 'wheeled' into the centre of the shell and would avoid abraiding any part of the shell with its extremely sharp point.

Specimens in which any of the cut edge of the shell had stained were rejected. The treatment of satisfactory specimens depended on the nature of the stain. The specimens were mounted without the use of any liquids in which those particular stains were at all soluble. Thus, shells which had been injected with water-soluble material, not soluble in or affected by xylene, were dried over phosphorous pentoxide and immersed directly in xylene. From this they were transferred to molten paraffin wax (60° C. m.p.), and cut in half along the long axis with a sharp scalpel while they were still embedded (the thin glass tube splinters during this operation, but does not usually upset the success of the experiment). The half-shells still embedded in wax were returned to xylene where the wax dissolved. The supporting material was brushed away and the halves were mounted in Canada balsam in cavity slides, with the orientation described above (p. 395). The exact depth to which stain had penetrated could then be followed. This procedure was adopted with basic and acid fuchsin, neutral red, ammoniacal silver nitrate, &c., before or after treatment with lipid solvents. Similarly, acids and alkalies could be injected before staining, to study their effect on the various layers. On the other hand, when shells were injected with lipid soluble stains, the material was frozen in glycerine jelly, cut in half and mounted in glycerine.

The existence of the amber layer was established from those experiments. It is permeable to small ions, and substances of small molecular size; examples are silver, copper, potassium, sodium, nitrate, chloride, bromide, and hydroxyl ions and to iodine.

On the other hand, neutral red and stains of larger size (basic and acid fuchsin, carmine, haematoxylin) do not pass through. However, the permeability to none of these substances is very great; silver would appear to be the biggest ion to pass through freely, while metallic ions (possibly because of their smaller size) penetrate more readily than acid radicles. Thus, concentrated potash does not attack either the resistant protein layer, or the soft protein layer which lies outside the ambe

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layer. But, about six hours after potash has been injected into an egg-shell with an almost complete exochorion, though no primary wax layer is on the inside of the shell, sufficient alkali has permeated the whole of the endochorion to dissolve the inner layer of the exochorion. This leaves the resistant exochorion as a detached sac around the intact endochorion. Since both resistant and soft endochorion protein layers (see below, p. 411) are readily permeable to water and water-soluble substances of all molecular sizes, and since there is no reason to believe that the discontinuous outer polyphenol layer has any more effect on diffusion than the inner one, selective diffusion must be attributed to this excessively thin lamina interposed between the outer polyphenol layer and the soft endochorion protein. A comparison of the above properties and those listed below with the characteristics of the amber material of the cap (p. 429) leaves no doubt that this is an amber layer identical with that in the cap, but invisibly thin. The amber layer of the cap is present between the outer polyphenol layer and the soft endochorion.

The penetration of picric acid through this amber layer is an interesting exception to the above phenomena. Its rate of diffusion is indeed so slow that unless the specimen is injected three times with the stain, coloration in the soft protein layer is insufficient to be conclusive. This is because the stain in the cavity dries in the space of two or three hours. (The injected shells cannot be kept in a very humid atmosphere to prevent this, since water condensing on the inside surface might allow staining solution to migrate to the cut edge and penetrate from there.) Picric acid is a larger molecule than others which will penetrate the amber layer, but as it is equally soluble in oil and water, it cannot be considered the largest water-soluble diffusant. It is probably its oil-solubility which carries it through the amber layer. (It is shown on p. 429 that lipid material forms the basis of the amber layer.)

The permeability of the amber layer is in no way altered, either by boiling shells of any stage in acetone, chloroform, benzene, or other wax solvents, or by treating either surface of the shell with strong wax emulsifiers such as I.C.I.'s C.09993

(Beament, 1945), and it is unaffected by potash in the cold, or strong nitric acid. Since it is neither waterproof nor impermeable to ions, it cannot be a wax layer (Beament, 1945, 1946 *a* and *c*).

When first formed, the soft endochorion protein (see below) does not seem to adhere to the amber layer, and, after fixation in aqueous Bouin, such part of the soft endochorion as has been secreted rips away with the fixed follicle, leaving the hydrofuge amber layer exposed. This may be due to the action of the fixative, for such separation cannot be brought about before fixation, and can be attributed either to unequal shrinkage or to the well-known effect of strong acetic acid on fresh protein. On the other hand, when shells with incomplete soft endochorions are stripped from their follicles, dried, and extracted in boiling chloroform or acetone for six hours, some separation along the amber layer takes place, though it is never complete.

We can conclude that the amber layer in the unspecialized region of the shell acts as a cement between the outer polyphenol layer and the soft protein layer, and that it is more strongly attached to the polyphenol substrate than the protein lying above it. This junction can be broken down by boiling lipid solvents soon after its formation, but before the exochorion is completed, the adhesion between the amber layer and the protein has increased so that its function as a cement is in no way altered by boiling lipid solvents.

#### SOFT ENDOCHORION LAYER

The follicle cells are now intensely basiphil, and after treatment with basic fuchsin, picric acid, and iodine, the whole cell stains, showing it to be crammed with protein material. The nucleus consists of large central bodies with many peripheral granules, indicating a phase of intense secretory activity (Text-fig. 4). The follicle deposits the final lamina of the endochorion which is the soft endochorion layer. In contrast to the preceding layers, it shows a structural relation to the morphology of the follicle cells almost from the beginning. Deposition of material takes place at a greater rate along the junctions between

individual follicle cells, and more slowly at the centres, so that by the time it is complete it is approximately  $10\mu$  thick under the cell borders, but only  $6\mu$  thick at the centre of each hexagon. Secondly, in contrast to the protein layer of the resistant endochorion, it shows slight irregular laminations. This is accentuated by staining after soaking halves of completed shells in water for forty-eight hours, or immersing in strong hydrochloric acid, boiling in saturated urea solution, &c. The lamination may be caused by slight lateral shifts in the follicle cells, probably initiated by changes of pressure transmitted by the tunica propria from the general body-cavity of the female, or by increases in the volume of oocytes above it in the ovariole. It should, perhaps, be pointed out at this stage that although an ovariole may contain up to six oocytes in various stages of yolk accumulation, and perhaps at its base one egg with a complete chorion (though not necessarily waterproofed), no ovariole was ever found in which more than one oocyte was in process of receiving its chorion.

In general chemical and physical properties, this layer is very similar to the protein layer of the resistant endochorion. It gives all the reactions listed on p. 405 for protein, and, in particular, Millon's reagent gives a faint pink coloration after heating, rather more slowly than in the resistant protein layer (contrast polyphenol islands staining in the cold).

When the layer is partially formed, and at all stages after its formation, it stains throughout its depth with the typical pink-brown of the polyphenol reaction after treatment with ammoniacal silver nitrate. With incomplete shells the membrane must be washed very rapidly in distilled water immediately after removal of the follicle and yolk in Ringer, and placed into the stain at once. This is necessary because in newly secreted material the polyphenol seems to be washed out by longer immersion in water. When halves of shells from which the larvae had hatched were soaked in water for twenty-four hours, or fixed in aqueous Bouin's solution, the amount of polyphenol staining was extremely slight; it was shown above that the staining of the resistant protein layer is slightly greater. It is thus certain that the bulk of the polyphenol is not fixed

in the protein layer. In order to confirm this, halves of freshly 'hatched' shells were treated with 5 per cent. silver nitrate solution; the whole endochorion took on a deep brown-black colour, showing the presence of quantities of silver reducing material. This reaction also diminished considerably after immersion in water. Similar half-shells were placed in 5 per cent. silver nitrate solution with the addition of nitric acid (a specific test for chlorides and bromides), and exposed to light for twenty-four hours. There was no darkening in any part of the endochorion of these half-shells greater than in controls in which the halves had been immersed in a similar concentration of nitric acid only (slight yellowness occurs, due to nitration). The endochorion does not, therefore, contain detectable quantities of salts such as sodium chloride, but it does contain a substance capable of reducing silver nitrate (which is not reduced by polyphenol), and which is slowly leached out by immersion in water. The most likely substance would seem to be a free amino-acid. It was subsequently found that Mörner's reagent gave a positive reaction with fresh, but not with water-leached material, showing that the untreated endochorion contains small quantities of tyrosine.

This may be correlated with the lack of pigment in the *Rhodnius* egg-shell. The dark colour of many insect cuticles and egg-shells is usually attributed to the presence of melanin pigments (Henke, 1924; Baldwin, 1940); tyrosine represents an intermediary product in their formation, and presumably the follicle cells of this particular insect are lacking in the necessary tyrosinase to complete the process (Cordier, 1928). The polyphenols in this layer may also be regarded as subsidiary chromogen for pigment formation (and see Wigglesworth, 1942). They are, however, present and distinct from the tyrosine. The two granular layers do not stain selectively in Mörner's reagent, nor do they react selectively to 5 per cent. silver nitrate. The distribution of the polyphenol material in this soft endochorion protein layer is not quite uniform. It is slightly more concentrated in the boundaries (next the amber layer below, and the exochorion above), and less in the central areas; there is no similar differentiation in the distribution of



tyrosine. We can conclude that the soft endochorion protein layer contains both diffuse, water-soluble, polyphenol and tyrosine elements, but that there is no detectable concentration of chloride or bromide present.

The soft endochorion is slowly soluble in strong nitric acid, and violently so in chlorated nitric acid. It is only very slowly attacked by concentrated aqueous potash; strong sulphuric acid is without effect, while concentrated hydrochloric acid merely induces slow swelling. When it is eventually broken down, no oil of any kind is released (see exochorion), nor will it stain with black Sudan B or Sudan III in alcoholic solution. Its resistance cannot therefore be attributed to lipidization, and it is very nearly identical in chemical properties with the protein layer of the resistant endochorion, though comparative less resistant to solution. Its resistance to attack by potash increases as the shell ages (see p. 406), while the layer is not attacked by trypsin or pepsin. It therefore consists of protein material slightly less tanned and 'hardened' than the protein in the resistant layer.

It may be noted here that both protein layers become almost jet black after immersing half-shells overnight in cold saturated *p*-benzoquinone solution, and the resulting tanned material is insoluble in everything but boiling chlorated fuming nitric acid, and fused potash at 140° C. Therefore, the material which forms the basis of the layers must be tannable protein, even after such 'hardening' as has taken place. A parallel state of resistance is shown by endochorion material fixed in picric acid, which is similarly resistant to acid and alkali, and will not take aqueous stains until it has been washed for several days.

The soft endochorion is probably the layer referred to previously as the 'endochorion'. This agrees with the properties described for the inner layer of the *Rhododendron* bug egg-shell (Johnson, 1934). It must be emphasized that the granules, which have been noted before in some eggs, are not merely discrete bodies dispersed within this soft material, but form a definite layer associated with the amber material. The inner (resistant) layer of protein material, although similar to that

in the outer layer, is a product of a separate secretory stage in the first phase of activity of the follicle cells, and the process of protein secretion is completely interrupted for the production of the intervening layers.

### Vitelline Membrane.

Throughout the deposition of the whole endochorion, the vitelline membrane of the oocyte can be readily removed from the inside of the egg-shell by a strong jet of water, or by careful scraping. It is a homogeneous layer, some  $2\mu$  thick, and stains very heavily in water-soluble protein stains, ninhydrine, &c. It is not, therefore, a waterproofing layer (and see Beament, 1946 *a* and *c*). It does not, however, contain any polyphenol or oil at any stage, and so does not apparently contribute to any of the processes of endochorion formation.

## THE UNSPECIALIZED EXOCHORION

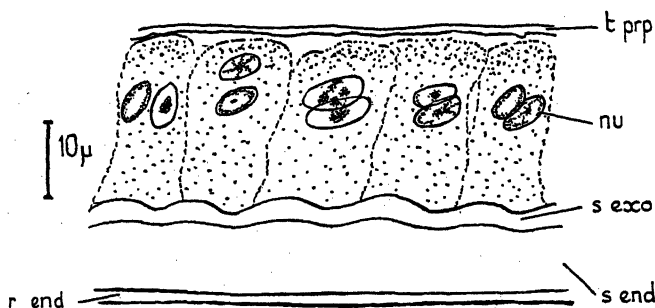
### State of the Follicle.

During the transitional period between the formation of the endo- and exo-chorions, the follicle cells undergo a marked change in staining properties. The cytoplasmic region no longer stains strongly with basic or acid fuchsin, borax carmine, or iodine, though picric acid staining may be even more intense. Though no discrete oil-droplets are visible, the cytoplasm stains rather palely with Sudan III and Sudan black B in 70 per cent. alcohol, and if follicle cells are placed in cold nitric acid, large quantities of oil are released which stain very deeply in the Sudan stains. At the same time the nuclei become smaller and the central bodies disperse in fine granules which move to the peripheral region (Text-fig. 7). These changes precede the secretion of exochorion material in every part of the shell (see p. 431, and Beament, 1946 *b*).

### The Soft Exochorion.

The material now secreted by the follicle cells forms a continuous layer over the undulating soft endochorion, and the initial region, approximately  $0.5\mu$  thick, is a uniform layer.

Beyond this thickness, however, the material is secreted at a greater rate over the ridges marking the cell boundaries, and decreases uniformly towards the central region where the rate of deposition is extremely small (Text-fig. 8). Thus, by the time a layer  $8\mu$  thick has been formed under the cell boundaries, the central region is about  $1\mu$  thick. Sections of the shell cut at  $8\mu$  (for method, see Beament, 1946 *b*) show that the central pit is filled with a long process from the follicle cell, and it



TEXT-FIG. 7.

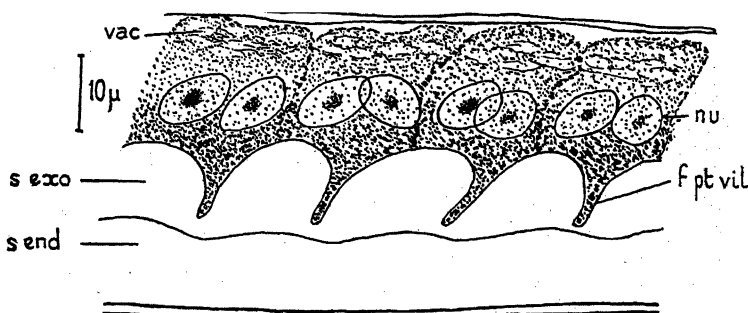
Longitudinal section through the unspecialized chorion, with follicle cells secreting the soft exochorion layer (*sexo*). *nu*, nuclei; *rend*, resistant endochorion layers; *send*, soft endochorion layer; *tprp*, tunica propria.

appears that since secretion takes place from the cell surface, the irregularity of deposition is exaggerated by taking place from the sides of this process as well as through the bottom. Material formed in the upper region of the cells will migrate downwards through the extended process, and will become less concentrated in the cell substance towards the tip.

The material secreted is translucent and colourless and is not laminated. It has the following properties:

If the chorion is removed from the ovary while this layer is still incomplete and is treated at once, it stains slightly with Sudan III and Sudan black B in 70 per cent. alcohol, and with picric acid. Basic and acid fuchsin and iodine give the layer very pale coloration, while there are weak xanthoproteic and Millon's reactions. There is no trace of polyphenol by the

ammoniacal silver nitrate test. This would indicate that while protein similar to that in the endochorion is present, it is modified by the presence of lipoid material. These properties are almost identical with those of the follicle cell cytoplasm which must, therefore, be crammed with preformed exochorion material. When first formed the chorion is still soft and flexible, but if the egg is punctured and the yolk contents



TEXT-FIG. 8.

Longitudinal section through the unspecialized chorion, with follicle cells secreting the resistant exochorion layer. Note the vacuolation (*vac*) of the follicle cells, and follicular pits filled by the villi from the cells (*fpt vil*). *nu*, nuclei; *s end*, soft endochorion; *s exo*, soft exochorion layer.

washed out and replaced by cotton wool, the layer hardens while drying in the air. If a similar specimen is left in Ringer's solution, the chorion becomes much more rigid than it is in a shell consisting of the endochorion only and treated in the same way.

However, after the exochorion has become hard, it will not give any of the staining reactions listed above, except with picric acid which does not stain rapidly or deeply compared with its action on freshly secreted material. On the other hand, even after the embryo has hatched, the soft exochorion dissolves rapidly in cold nitric acid and in cold potash, with the liberation of large quantities of oil which will take up Sudan stains. Despite this large lipoid component, the properties of the soft exochorion material are in no way altered by extraction

for long periods in boiling lipid solvents such as chloroform, benzene, acetone, &c., nor is any oil accumulated in the solvent. The lipid must, therefore, be chemically attached to the protein component present.

The maturation of the soft exochorion material described above must represent a different change from that which takes place in the soft endochorion. If the chemical resistance of the endochorion (see p. 406) is due to tanning, and possibly vulcanization, then such agents would appear to be lacking in the soft exochorion where protein is mostly modified by the presence of lipid. However, concentrated hydrochloric and sulphuric acids do not dissolve the soft exochorion, nor is it attacked by trypsin or pepsin; it is, however, dissolved by diaphanol in the course of eight weeks.

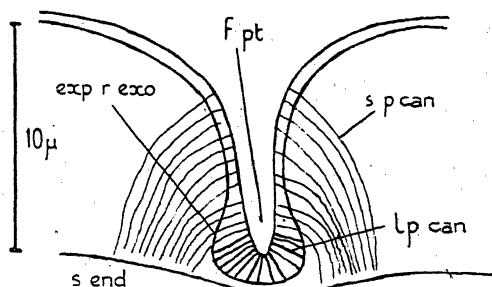
The soft exochorion is, therefore, probably composed of a lipoprotein preformed in the follicle cells and secreted as such (contrast the amber layer, p. 430). It is probably the material which previous authors have wished to indicate when they referred to the chorion as being composed of 'chorionin' (Tichomiroff, 1885, &c.), and its properties are very similar to those of the cuticulin layer of the insect epicuticle (Wigglesworth, 1946). It is, therefore, proposed to limit the term 'chorionin' to the preformed lipoprotein in the material of the insect egg-shell.

The soft exochorion is rapidly tanned to a dark brown colour by overnight immersion in cold saturated aqueous solutions of *p*-benzoquinone. After such treatment, or after immersion for long periods in picric acid, its solubility in potash and in nitric acid is considerably reduced. In this respect it is very similar to the soft endochorion. The protein of the exochorion has not, therefore, been modified by the lipid component, to the exclusion of this type of 'tanning' process (see amber layer, p. 430).

#### Resistant Exochorion.

The final phase in chorion formation consists in the secretion of the resistant exochorion, which again covers the whole shell. During this process the follicle cells become flattened and more

shrunk, while irregular vacuolation appears in the outer regions; these are signs of the approaching necrosis of the cells (Text-fig. 8). The cells do not, however, differ from the preceding phase in their nuclear structure or cytoplasmic staining. The material added gives the shell surface its final sculptured appearance. It is a thin layer, less than  $1\mu$  thick over the

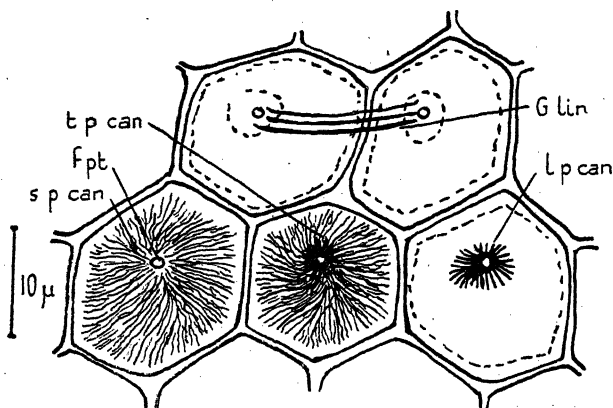


TEXT-FIG. 9.

Diagrammatic section through an unspecialized follicular pit (*fpt*) in the completed chorion, after silver bromide treatment, showing large (*lpcan*) and small (*spcan*) varieties of pore canal, and the expanded region of the resistant exochorion layer (*exp r exo*). *send*, soft endochorion.

regions corresponding with the boundaries of the follicular cells, but increasing regularly in thickness over the walls of the follicular pits and expanding over their tips so that it is almost  $2\mu$  thick at this point (Text-figs. 2 and 9). It could not be distinguished from the soft exochorion layer by any staining properties. Its boundary is not delineated by any line of junction, and there is no visible difference in optical density between this material and that of the soft exochorion. It is differentiated by its solubility, for it is not readily attacked by warm nitric acid, nor by cold concentrated potash. It can, therefore, be obtained by placing fragments of completed shells in cold potash, when it separates as a discrete layer. It is, however, broken down by boiling nitric acid and by cold chlorated nitric acid, and when this takes place oil is released, as with the soft layer. The proportion of oil present may be greater than in the soft layer, though less than in the amber

layer (see p. 430). It is soluble in diaphanol in eight weeks. There are no indications of polyphenol material during its formation, and after treatment with cold *p*-benzoquinone it is indistinguishable from the soft layer. It is probably a more highly polymerized form of chorionin which is the basis of both layers.



TEXT-FIG. 10.

Diagram showing the distribution of pore canals in the unspecialized chorion, seen in surface view. *fpt*, site of follicular pit; *Glin*, 'Galliard' lines; *lp can*, large pore canals only; *sp can*, small pore canals only; *tp can*, all pore canals; the dotted lines indicate the boundaries of the pore canals.

### Follicular Pits.

Each pit in the exochorion (Text-figs. 2, 8, and 9), which contains the long process of a follicle cell during chorion secretion, is approximately  $8\mu$  deep; the upper part forms a funnel leading to a tube  $2\mu$  diameter and  $5\mu$  long. These tubes depart from a position vertical to the plane of the shell, and this varies over the surface of the unspecialized region. Thus, at the rear end of the egg (Text-fig. 11) they are radial to the curved surface, but over the central region the blind ends point more towards the rear end. At the neck (Text-fig. 12), however, they are again radial. If the follicle is being subjected to pressure from the growing oocytes above, so that the rear end of the egg is pushed into the closed base of the ovariole, the

outer ends of the follicle cells will undergo a slight upward displacement which will be continuous throughout the process of exochorion secretion, and this will account for the inclination of the pits.

### Pore Canals.

When the outer surfaces of completed shells, stained with ammoniacal silver nitrate, were examined, it was found that the area of each polygon occupied by the funnel and tube of the follicular pit was marked by a number of evenly distributed black dots, less than  $1\mu$  in diameter, which were not present over the borders of the hexagon. Occasionally these appeared to extend for a very short distance (less than  $1\mu$ ) as a broken line into the depth of the exochorion substance. Also, when sections of the almost complete shell, fixed in aqueous Bouin's solution and stained with basic fuchsin, were examined under the highest magnification, it was found that very occasionally the faintly staining cytoplasm of the follicular villus was continued into the side of the follicular pit as a series of minute granules, extending linearly for 1 or  $2\mu$ . This contrasts with the exochorion material which does not stain at all after Bouin fixation.

Added to this, despite the lipophilic nature of chorionin, the egg is not markedly more waterproof after the addition of the soft exochorion, and some eggs were obtained which had been released from their follicles into the calyx, but which had a similar high rate of water-loss (see Beament, 1946 *a* and *c*).

It appeared, therefore, that the exochorion might be porous, and that its method of formation might be similar to that of many biological materials (insect cuticle, Plotkinow, 1904; Kühnelt, 1928; bone, Murray, 1936), i.e. by secretion around numerous fine cytoplasmic filaments thrown out by the cell surface. An extensive search for such structures was therefore made. Pieces of shells at all stages of formation were treated by the rapid Golgi process, and were heated in air and plunged into canada balsam (Wigglesworth, 1933). Neither method gave any indication that fine canals were present in any layer. The following method was, therefore, adopted:



Halves of shells were immersed in saturated potassium bromide solution for three days, so ensuring that any spaces in the shells would be filled with the liquid. They were removed, external liquid was taken off by drying rapidly on filter paper, and they were placed in the dark in a large excess of a 5 per cent. solution of silver nitrate for a further three days. This would cause a precipitate of silver bromide in all regions of the shell capable of penetration by silver and bromide ions, and in any cavities. The white precipitate of silver bromide was brushed away from the shell surfaces in distilled water, and hand sections cut from the shell fragments. These were mounted in glycerine and developed in the light of a bench lamp. This process revealed a series of fine brown filaments emanating radially from the sides of each follicular pit, and, where their whole length could be traced, running down to within  $1\mu$  of the upper surface of the soft exochorion layer. They were apparently open on the surface of the resistant exochorion, but never penetrated to the endochorion layers, nor was there any sign of similar structures within any of the endochorion layers. The pores appeared to follow curved paths (Text-figs. 9, 10), and were divisible into two main types; long ones running from the sides of the funnel of the follicular pit, measuring less than  $0.2\mu$  in diameter, and short ones running through the bulb-like expansion of the resistant exochorion around the lower tip of the pit, which were about twice as thick. In surface view they seemed to be more concentrated along those radii of the polygons which passed through the angles, rather than to the centres of the sides of the polygons, though this may have been selective staining or due to the greater distance which these traverse. There were altogether about four-hundred canals attached to each follicular pit.

Similar structures were found in other regions of the shell, always confined to the exochorion layers, and will be described under those areas (and see Beament, 1946 b).

The surface of the exochorion is not readily wet when eggs are placed on the surface of water, though they are more dense than the supporting medium. However, after immersion in distilled water for a few moments, the surface is made much

more hydrophilic, probably through water molecules adsorbed on to the surface, and eggs so treated sink at once when they are dropped on to water. Since water can penetrate along these canals, and probably this will take place easily as soon as the surfaces of the canals have adsorbed a complement of water molecules, it is very important to discover whether water or other substances can pass through the canals from the outside of the shell to the endochorion layers. The exochorion does not appear to waterproof the egg, and this at first seems to be due to the pores.

If eggs, before waterproofing, are placed in dilute solutions of stains, the chorion acts as an osmotic membrane and they swell up and burst. A solution of basic fuchsin was therefore made up by saturating Ringer's solution with the solid stain, and eggs of all ages were immersed in this. Until the endochorion is complete the soft endochorion stains deeply and rapidly from the surface, so that it is uniformly stained in 30 minutes. On the other hand, as soon as a layer of exochorion material  $0.5\mu$  thick has been formed, the endochorion is completely unstained after immersion for two hours, and, if left in the stain for longer intervals, the slightest staining takes place at the neck, due to stain entering at the rim and migrating through the endochorion from there (see Beament, 1946 *b*). The pore canals are certainly large enough to permit the penetration of stain molecules, but they may form small air-locks which prevent the penetration of the staining solution. On the other hand, alcohol wets the shell surface readily, and so alcohol saturated with basic fuchsin was used as a stain.

A glass apparatus was constructed, using two chambers, each of approximately 1 c.c. volume. These were separated by a ground-glass tap, and a further tap gave access to each chamber. Eggs of various stages (dissected from the ovaries) were placed in one chamber; the other was filled completely with the solution, and the taps of this chamber were closed. The chamber containing the eggs was attached to a 'hy-vac' pump, evacuated, and sealed. Then the tap between the two chambers was opened so that the eggs were completely immersed in stain which could fill the pore canals without any air-locks, but no

stain penetrated the endochorion layers through the unspecialized exochorion.

It was then obvious that the pore canals do not open on to the surface of the endochorion, and that the exochorion material must be permeable to water since it does not waterproof the egg with its innermost continuous layer. When totally immersed in ammoniacal silver nitrate the endochorion layers are not stained, so that the layer has a very small molecular pore size for diffusion, and, as with the amber layer, material which is also oil-soluble (such as picric acid) must pass through by solution rather than by diffusion.

The pore canals of the *Rhodnius* egg-shell are, therefore, primarily a result of the method used for the secretion of the exochorion. They are imperforate at the lower ends, and could not be used to convey material to the endochorion after the exochorion has been formed. They are not associated morphologically with the differentiation between the soft and resistant exochorion layers. During exochorion formation they are filled with cytoplasmic filaments, and the outer parts of them, which stain black in ammoniacal silver nitrate, may contain residues of this cellular material. When the egg is laid they are covered by the cement substance and so may not play any vital part in the physiology of the developing embryo, other than possibly increasing the rate of diffusion of air through the shell, but this effect would be very small (Beament, 1946 b).

The parallel structure of the insect cuticle may be compared at this point, for Wigglesworth (1946) has shown that, in *Rhodnius*, pore canals filled with cytoplasmic filaments penetrate through the endo- and exo-cuticles, and open at the surface of the epicuticle lipoprotein layer. In the past there has been considerable controversy over the function of these canals. Among insects, Wigglesworth has shown that they have an important role in secreting and repairing the epicuticle wax layer. In the egg-shell, their main function is restricted to secretion, but it is interesting to note that in the endochorion layers where protein material is deposited and further materials added to modify their properties, no pore

canals are present, whereas they form part of a secretory system in which the material deposited is apparently completely pre-formed in the follicle cells. This is, at first, contrary to expectations, for they would be an ideal agent for impregnating a thick layer with an additional secretion. Their presence in the lower layers would, however, render the egg contents very vulnerable to the entry of material deposited on the shell surface.

It is also interesting to note that the pore canals are distributed in a regular pattern, being confined to the central region of each polygon corresponding to a follicular cell. This is directly comparable with the distribution of canals in certain insect cuticles (Hass, 1916; Kapzof, 1911) where pore canals are again distributed in bundles, each related to a cell of the insect epidermis.

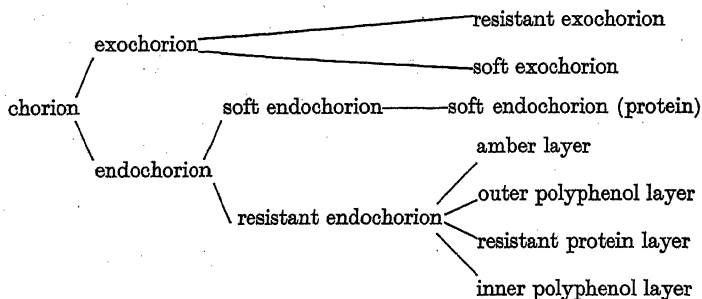
#### The 'Galliard' Lines.

Galliard (1935), in his study of the reproduction of *Triatomids*, makes no mention of pore canals in the shell of *Rhodnius* eggs, but does figure a peculiar 'spike' attached to the tip of the follicular pits in the ventral region only of the unspecialized shell. According to his drawings these 'spikes' would appear to penetrate through most of the endochorion layers. A search for these structures revealed that differentiation of the ventral region could be obtained only after treatment with absolute alcohol during any staining process. The 'spikes' do not emanate from the tips of the follicular processes, and Galliard must have confused them with pore canals. They take the form of one, two, or three thin lines of material lying wholly in the exochorion, and about  $2\mu$  from its outer surface. They appear to run from one polygonal unit to the next along the length of the shell (Text-fig. 10), and are the only form of connexion between such units. They are not definitely shown by the silver bromide method for pore canals, and they may be considered as artifacts due to the action of alcohol. No function can be attributed to these structures at present. They may possibly mark the presence of the transecting bar running between the two halves of the follicular bicells.

## CONCLUSION

## Unspecialized Chorion.

The complete distinction between the endo- and exo-chorions outlined on p. 397 is now apparent (Text-fig. 2). They differ in their mode of formation and structure as well as in chemical properties, though all the layers are apparently modifications of a protein material. Before passing to the specialized areas of the shell, the unspecialized chorion may be summarized as follows:



## SPECIALIZED AREAS OF THE SHELL

The structure of the shell can be visualized as a series of rhombohedra of material, each the product of a single follicle cell, and whose seven layers are confluent with those of the surrounding units. In describing and discussing the specialized areas of the egg-shell, the following principle will be found to apply:

Differentiation is carried out by variations in the thickness of the seven layers, and such variation may be large between successive rhombohedral units, but is usually much smaller within a single unit.

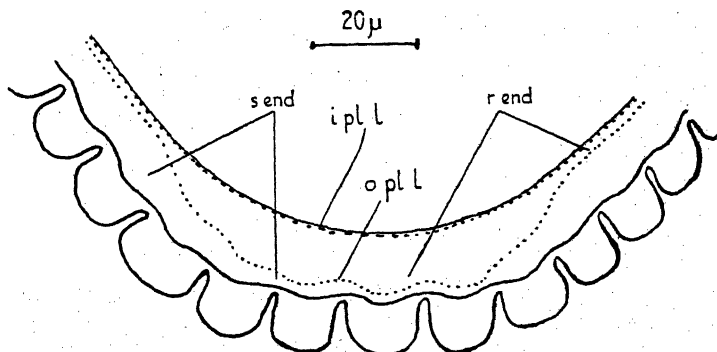
## The Rear End of the Shell.

The shell at the rear end of the egg is slightly modified; the resistant endochorion layer is expanded to over half the thickness of the endochorion, while the soft protein layer is

correspondingly reduced (Text-fig. 11). Slight modifications in structure have been reported in the rear end of the egg of the Rhododendron bug, *Notostira* (Johnson, 1934), and in the egg of *Nepa cinerea* (Köhler, 1906).

### The Neck of the Shell.

Between the anterior end of the unspecialized shell and the rim of the shell there is a short annulus of the chorion which is comparatively thin. The egg-shell is slightly constricted at this



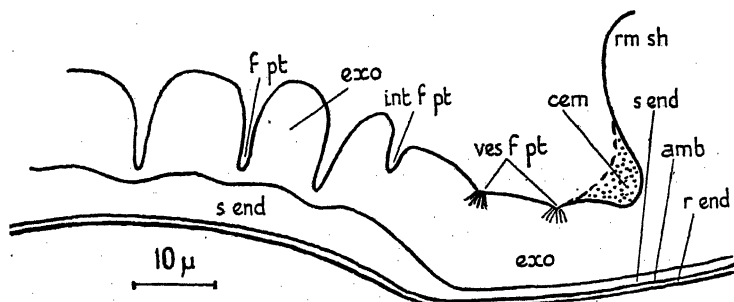
TEXT-FIG. 11.

Diagrammatic section through the rear end of the shell, showing the displacement of the outer polyphenol layer (*opl*) towards the soft exochorion layer. *ipl*, inner polyphenol layer; *rend*, resistant endochorion layer; *send*, soft endochorion layer.

point, before it expands into the rim. This is the neck, which differs from the main shell in the following characteristics (see Text-fig. 12).

The whole of the resistant endochorion, including the amber layer, is identical with that of the main shell, and is secreted at the same time. The follicle cells are indistinguishable when they start to secrete the soft endochorion, but after a layer between 1 and 2  $\mu$  thick has been formed, three rings of follicle cells around the egg change to the non-staining phase in which lipoprotein is secreted, and commence the production of soft exochorion material. The layer of soft endochorion

material is too thin to show the irregularities produced by the uneven distribution of secreted material (p. 416), and so the substrate on to which the soft exochorion is laid is plane. The soft exochorion material is also unevenly distributed, but until the thickness of this layer has reached about  $7\mu$ , the difference between the centres and sides of each follicular polygon is only about  $2\mu$ . Secretion takes place very slowly, correlated with the fact that the only pore canals found in this



TEXT-FIG. 12.

Longitudinal section through the neck region, showing the modifications in the chorion structure. *amb*, amber layer; *cem*, typical appearance of a cement deposit; *exo*, exochorion; *fpt*, normal follicular pit; *intfpt*, intermediate follicular pit; *rend*, resistant endochorion layer; *rmsh*, rim of shell; *send*, soft endochorion layer; *vesfpt*, vestigial follicular pits.

region are 'vestigial' bunches running from the centres of these small depressions and penetrating about  $2\mu$  deep (Text-fig. 12). Thus, the soft exochorion layer in the neck is of the same thickness as that layer in the main part of the shell, but it is completed during the time in which the main shell cells have produced a soft exochorion and an additional  $8\mu$  of soft endochorion protein; resistant exochorion formation takes place simultaneously in both places. A further ring of follicle cells forms the transitional region between the neck and the unspecialized shell. At this point the soft endochorion tapers fairly sharply, and while the exochorion is of the same thickness, the inner ends of the follicular pits formed by these cells penetrate only about half-way across the soft exochorion.

### The Cap.

The cap of the *Rhodnius* egg is a thin, slightly convex disk which is displaced to allow the larva to emerge. It is divided into two regions: the thick rim, which is described by Beament (1946 b), and the central region composing most of this area and described below.

When chorion formation starts, the follicle cells over the anterior region of the egg differ from the remainder only in their rather greater length, and by the fact that the area eventually covered by the cap is slightly flattened. The resistant endochorion is secreted contemporaneously with the rest of the shell, but is much thinner and it is difficult to resolve two distinct layers of polyphenol islands in section under the highest magnification. Islands of both dimensions are, however, present, though they probably make contact through the resistant protein material in some places (Text-fig. 13 c). Following this, a layer approximately  $2\mu$  thick is secreted, and this has all the chemical properties of the freshly formed soft endochorion protein layer (see p. 412). The layer is slightly thicker towards the rim and thinner at the centre of the cap. It is composed of translucent material, and is readily soluble in concentrated potash and strong nitric acid. But, during this secretory phase, large oily droplets accumulate in the distal regions of the cap follicle cells, staining strongly in Sudan III, Sudan black B, and B.Z.L. (Ciba). This is the only occasion when discrete oil droplets can be distinguished in the follicle cells, or when any lipid material is discernible in cells during the phase of endochorion secretion. The disappearance of these droplets corresponds with a complete change in the appearance of the protein material which has been secreted. It becomes a pale amber colour and, after drying, is very rigid and brittle. If caps of eggs in the early stages of chorion formation, but with an amber colour, are placed in cold concentrated potash, the amber material remains and is not readily dissolved by boiling the solution. The amber layer is similarly not attacked by cold 'chlorated' fuming nitric acid, but when heated in this solution it does break down with the evolution of an enormous amount



of oil which would seem to be equivalent to its own volume. As soon as the amber material has been formed its properties cannot be changed in any way by extraction for long periods in boiling lipid solvents such as acetone, chloroform, and benzene, nor can any lipid be obtained in the extract. Its surface is extremely hydrophobe and is not altered by long immersion in water.

By injecting materials into the anterior end of the shell after various treatments, it was shown that the amber layer is permeable to substances of small molecular size such as water and iodine, and to potassium, sodium, silver, nitrate, chloride, bromide, and hydroxyl ions. It is impermeable to neutral red and water-soluble stains of large molecular size, but permeable to picric acid (which has appreciable oil solubility as well). It is not stained by either Sudan III or Sudan black B in 70 per cent. alcohol. (These two stains were applied to fragments of caps so that the stain could penetrate through the cut edge of the amber material.)

This layer is identical in properties with the sub-microscopic layer outside the outer polyphenol layer of the unspecialized shell (see p. 407) which was called the amber layer, although it was invisibly thin. It occupies the same position in relation to the resistant endochorion and to the layers above it in both regions of the shell. In the unspecialized shell region it might be considered that the minutely thin amber layer was merely a vestigial remainder of the thick layer in the cap. It is, however, complete over the whole shell despite its thinness, and is the only barrier to water-soluble material of larger molecular size in the inner layers of the shell.

The amber layer consists, therefore, of protein, probably with added tanning and associated agents, which is 'lipidized' after secretion, thus distinguishing it from the preformed lipoprotein or 'chorionin' of which the exochorion layers are composed. It is far more resistant to chemical attack than is chorionin, possibly because such tanning and hardening elements as are present in endochorion protein are added before the lipid. If the polyphenol content of material is any indication of its tanned nature, the preformed chorionin shows no

such reactions at any stage in its formation, while its rapid solubility in cold potash indicates that very little tanning can have occurred. Also, the parts of the protein molecule which take part in reactions with *p*-benzoquinone are not blocked by the presence of lipid in chorionin, whereas amber material is not greatly darkened by immersion in quinone solution.

One further point occurs. Since the protein of the amber layer (before lipidization) is apparently indistinguishable from the protein layer of the soft endochorion, it is difficult to understand how this underlying layer escapes lipidization when the oil is poured in. Possibly a substance has already been placed in the resistant protein layer preventing the protein from being lipidized; otherwise, the minute amount of oil added to form the amber layer of the unspecialized shell would never form such a complete, uniform, and discrete layer.

The amber material is not attacked by diaphanol after nine weeks immersion which is sufficient to break down all the other layers of the chorion. It is, however, destroyed by placing in fused potash at 140° C. for one hour. Diaphanol is supposed to dissolve all biological materials except chitin, and there is no evidence that the amber layer contains any chitin. This apparent discrepancy in the action of diaphanol may to some extent explain reports that insect egg-shell contains chitin.

As soon as the amber layer has been formed, the histology of the follicle cells changes and they have the typical appearance and properties of cells in the second (or exochorion) secreting phase. This transition takes place slightly before the similar changes in the cells of the neck, and long before the change in the unspecialized shell cells. But, during this time, a minute amount of soft protein material is deposited over the amber layer. This represents the thick soft endochorion protein layer of the main shell. Its presence was shown in the following way:

When whole caps were immersed in stains such as basic fuchsin they were quite unstained, but when they were cut in half before staining and the cut edge observed with an oil immersion objective, a thin deeply staining line was detected between the amber layer and the non-staining exochorion above. This thin lamination was shown to have all the

properties of the soft protein material; its thickness is very irregular, at some points being about  $1\mu$  thick and at others seeming to disappear completely. On the average, however, it is thicker towards the centre of the cap and thinner towards the rim. These irregularities do not correspond to the follicular pattern.

The surface on to which the cap-cells secrete the soft exochorion lipoprotein is, therefore, composed of soft protein, and though uneven has no ridges on which the sides of the follicular pits can be built up. Secretion of soft chorionin takes place very rapidly, and at a much greater rate beneath the cell borders than at their centres, so by the time the central region is about  $4\mu$  thick the sides are about  $16\mu$  thick (almost the complete thickness of the cap exochorion). The process is, however, by no means as regular as it is over the main shell (see Text-fig. 13 A). The sites of the follicular pits of the cap are marked by large irregular cavities filled by cell processes, but the entrance to each cavity is often constricted. The sides of these holes are penetrated by numerous pore canals, but none is present on the exposed upper surface outside them. The cavities decrease in size more by material added to the sides and around the orifice than to the bottom, so that by the time the resistant exochorion layer is secreted, the follicular pits have assumed their final shape. The final layer (see Text-fig. 13 A) is 1 to  $2\mu$  thick over the exposed surface of the cap, but is thickened where it lines the follicular pits. Thus, it is approximately  $4\mu$  thick at the neck of each pit and  $2\mu$  thick over the inner surface.

In appearance the pits in the cap differ markedly from those of the main shell region (Text-figs. 2 and 13 B). Each opens on to the surface by a slit-like aperture, the direction of the slit bearing no relation to its position on the cap. From the narrow orifice it expands into a cavity approximately  $3\mu$  across and about  $8\mu$  deep, and, in contrast to the main shell, there is, thus, a considerable depth of soft chorionin between the bottom of the pit and the endochorion layers. The inner ends of the pits are not surrounded by bulb-like expansions of resistant exochorion material; they are bluntly pointed, and occasionally forked or completely irregular (Text-fig. 13 A).

Pore canals are present only in the exochorion layers of the cap, as in the shell (Text-fig. 13 B). Their outer openings are restricted entirely to the surface of the pits, and none is detectable in the exposed surface. Two types were found, as in the main shell; long fine ones which run from the expanded cavity of the pit towards the endochorion, but, as before, do not open on to it, and short thicker ones which are concentrated into a 'ruff' around the narrowest part of the orifice. These short canals do not penetrate through the resistant exochorion, which is expanded in this region. It may be noted that similar thick short pore canals occur in the main shell at the lower tips of the follicular pits where the resistant exochorion is also thickened. They may, therefore, be associated with the formation of a thickened region of the resistant material.

We can conclude that while the morphology of the cap is totally different from that of the shell, identical materials are present and are secreted in the same order. Differentiation is brought about by variation in the relative proportions of the components of the chorion and not by any modifications in the type of secretion (Text-fig. 13 c).

With the completion of the chorion layers, the plug of cells at the base of the ovariole breaks and the egg is released from the follicle, which has removed all its cytoplasmic villi. The necrotic remains of the follicle cells become the corpus luteum.

#### DISCUSSION ON THE INNER SURFACE OF THE CHORION

The inner polyphenol layer forms the substrate on to which the primary wax layer is added at a later date (Beament, 1946 *a* and *c*). The polyphenol-impregnated surface of the insect epicuticle is apparently a homogeneous substrate, at least in so far as the even distribution of polyphenols is concerned (Wigglesworth, 1945, 1946). The granular and discontinuous nature of the inner polyphenol layer must, therefore, be considered.

Wigglesworth (1946) has shown that in insect cuticle the polyphenols are secreted in a liquid medium, and appear as isolated droplets at the upper ends of the pore canals passing through the epicuticle lipoprotein layer. At such a stage, the

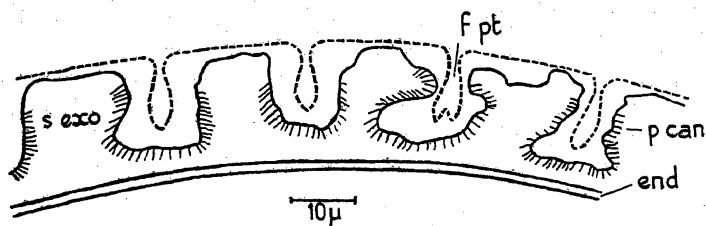


FIG. 13 A

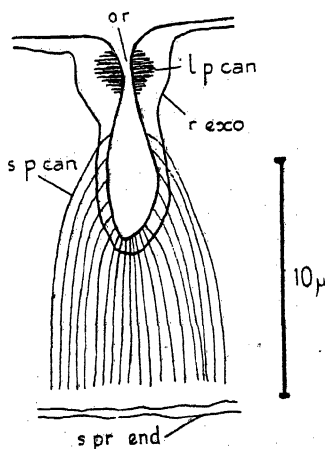


FIG. 13 B

TEXT-FIG. 13.

A, section through the central region of the cap during secretion, showing the large irregular pits, and pore canals (*p can*) and (dotted line) the shape of the follicular pits in the completed cap. *end*, endochorion layers; *s exco*, soft exochorion. B, section through a follicular pit of the cap, after silver bromide treatment. *l p can*, large variety of pore canal; *or*, orifice of pit; *r exco*, expanded resistant exochorion layer; *s p can*, small variety of pore canal; *s pr end*, soft endochorion protein layer. C, diagram comparing the relative thicknesses of layers of the cap with those of the shell. *amb*, amber layer; *ipll*, inner polyphenol layer; *opll*, outer polyphenol layer; *rend*, resistant endochorion protein layer; *rexco*, resistant exochorion layer; *s end*, soft endochorion protein layer; *s exco*, soft exochorion layer; *lw*, primary wax layer.

distribution of the polyphenol material over the epicuticle is very similar in appearance to the inner polyphenol layer of the chorion, though the droplets in the cuticle are roughly hemispherical. But these droplets contain tanning and other agents (as well as the polyphenols) which may allow the liquid droplets to wet and spread over the lipophilic cuticulin layer of the

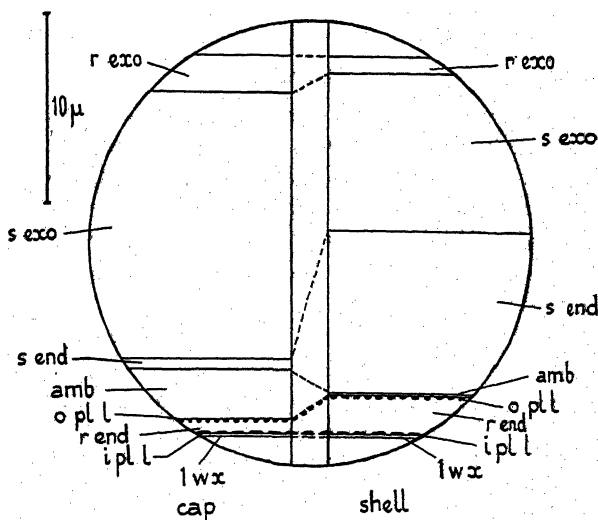


FIG. 13 c (see opposite)

cuticle, forming a continuous layer. The volume of materials secreted is apparently sufficient to run together and form such a layer.

On the other hand, when the inner polyphenol layer of the chorion is secreted, the substrate is the hydrophilic vitelline membrane of the egg on the inside, and the follicle cell membrane on the outside. If this material wetted the vitelline membrane, it would undoubtedly impregnate and tan it, and would not form a polyphenol layer of any kind. But it has been shown above (p. 415) that the vitelline membrane does not contain polyphenols at any stage. Therefore, if the material which forms the polyphenol granules is secreted in liquid form (and secretions which are actually solid when they pass through the

cell-wall are almost unknown), it will not spread over the hydrophilic vitelline membrane, but on the contrary collect in small hydefuge droplets and harden as discrete islands, especially as the amount secreted is small. These will be flattened by being compressed between the vitelline membrane and the follicle cells, which will give them their irregular outline. Such phenomena fit admirably with the final appearance of these islands.

### SUMMARY

The main regions of the *Rhodnius prolixus* egg-shell have been defined; a concise definition has been obtained for the term 'chorion'. The formation and structure of the unspecialized chorion has been followed from the time of differentiation of the follicle cells, up to the completion of the shell, and an assessment made of the chemistry and permeability of each component shell layer.

The follicle cells are binucleate; changes in morphology and histology prior to secretion of the shell are outlined.

The secretory products of the follicle cells fall naturally into an endochorion and an exochorion; the endochorion consists of five modifications of a proteinaceous substance. They are, in order of secretion:

1. The Inner Polyphenol Layer, which consists of a series of tanned granules of average diameter  $2\mu$ , containing large quantities of polyphenols. The layer is discontinuous and has no effect on permeability.

2. The Resistant Protein Layer, a tanned and possibly vulcanized layer of protein, 1 to  $2\mu$  thick, containing diffuse polyphenols. It is resistant to strong acids and bases, and permeable to water, ions, and large water-soluble molecules.

3. The Outer Polyphenol Layer, which is similar to the inner layer, but has more minute granules.

4. The Amber Layer.—This is the only coloured layer of the shell, and is less than  $0.1\mu$  thick. It consists of tanned protein to which oil is added after secretion. It is therefore a lipidized protein, which is excessively resistant to acids and

alkalies, and permeable to oils and oil-soluble material and to small ions and water.

5. The Soft Protein Layer.—This is a thick laminated layer some  $8\mu$  thick, similar to, but less resistant than, the resistant protein layer. It contains polyphenols and tyrosine. The layer is freely permeable to water-soluble substances.

Throughout the secretion of the endochorion, the follicle cells stain deeply and appear to be filled with the protein components of the shell.

The exochorion consists of two layers of the lipoprotein 'chorionin'.

6. The Soft Exochorion Layer is a lipoprotein which is soluble in potash but not in strong acids; the layer is permeable to lipid solvents and to water and small ions, but not to larger particles. It is  $8\mu$  thick at its maximum thickness, but contains follicular pits which, during secretion, are filled by long processes from the follicle cells.

7. The Resistant Exochorion Layer is a more resistant form of chorionin. It lines the pits and covers the surface of the shell, giving rise to the polygonal markings corresponding to the follicle cells, each with a pit at its centre.

The follicle cells contain quantities of lipoprotein during this phase of secretion, and are difficult to stain.

A method of staining is described which shows that pore canals of two varieties are present in the exochorion layers only. They run from the walls of the pits but do not reach the endochorion.

None of the layers of the chorion waterproofs the shell.

In the rear end of the shell, the outer polyphenol layer is displaced towards the exochorion, thus increasing the resistant protein layer and reducing the soft protein layer.

It is shown that all seven layers are present in the neck, and in the central region of the cap, and that the order of secretion is the same. Modifications are produced by variations in the thickness of the various layers.

In the neck, the soft protein layer is reduced; in the cap, the



resistant protein layer is reduced while the amber layer is  $2\mu$  thick, giving the cap a brown appearance. The soft protein layer is extremely thin and irregular while the exochorion layers are  $16\mu$  thick. Pore canals are again present in two varieties.

Some analysis is made of the formation of follicular pits; this appears to be correlated with the thickness of the exochorion and endochorion layers.

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# The Histochemical Recognition of Lipine

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With Plate 10

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## INTRODUCTION

ALTHOUGH lipines are constant constituents of cells, very little is known about their significance in vital processes. In order to learn something about their rôle, it is necessary first of all to be able to locate them in the cell. Kaufmann and Lehmann (1926 *a* and *b*), who made a very thorough study of the histochemical methods for showing various lipoids, found that the Smith-Dietrich test was the most reliable of all for indicating the presence of lipine. It will be recollected that this test is a modification of Weigert's method for myelin. Weigert's method was used by Smith and Mair (1908) for colouring lipoids other than myelin, and Smith (1909) subsequently undertook

an analysis of the principles of the method. The test is usually performed according to the instructions of Dietrich (1910).

In the Smith-Dietrich test, tissues are fixed in formaldehyde solution, frozen and sectioned. The sections are treated with a warm solution of potassium dichromate, stained with Kultschitzky's haematoxylin (also warm), and differentiated with Weigert's borax-ferricyanide. They are mounted in laevulose.

The Smith-Dietrich test has three main drawbacks:

(1) It gives a negative result with pure lecithin, as Kaufmann and Lehmann showed.

(2) It is not very sensitive. It fails to show lipine when present in small cell-inclusions.

(3) The period of differentiation of the stain is 'über Nacht'. By varying one's opinion as to the duration of that period, one can get positive and negative results with the same object.

The purpose of this paper is to present two new histochemical tests, called the acid-haematein and pyridine-extraction tests. The former is so called because positively reacting substances are coloured (blue, blue-black, or grey) by haematein used in acid solution. The test is based on that of Smith and Dietrich, but differs from it in giving a positive reaction with lecithin, in being more sensitive, and in avoiding all uncertainty due to ambiguous directions. The test gives a positive reaction with mitochondria, while the Smith-Dietrich does not.

The acid-haematein test gives a positive reaction not only with lipines, but also with certain proteins; and a second test, the pyridine-extraction test, is therefore performed in order to distinguish between the two groups of substances.

In this paper the word 'lipoid' is used in its widest physical sense, to include fats and all substances that occur in plants and animals and resemble fats in solubility. The word 'lipine' refers to lipoids that yield fatty acids, phosphoric acid or galactose, and a basic nitrogen compound. (Phosphatidic acids are not considered in this paper.) The words lecithin, cephalin, sphingomyelin, and galactolipine are used as group-names for the kinds of substances classified under these heads.

## THE ACID-HAEMATEIN TEST

## The Method by which the Test was Devised.

In order to devise a test that would separate lipines from all other substances, it was necessary first to work with the pure substances themselves. Kaufmann and Lehmann adopted the plan of incorporating substances in pieces of pith and then treating the pith as though it were tissue. This is satisfactory for some substances, but I soon found that it would not work with others. Some substances are difficult to draw into the cells of pith, while others fall out directly the sections are made; and thin sections cannot be cut. I therefore sought some other way of applying histochemical tests to pure substances. It is essential to apply tests to very thin layers, for a test that would be excellent macroscopically might be useless with minute objects. For instance, one might get quite a deep colour-reaction with a certain substance in a test-tube; but if mitochondria were formed of that substance, one would have to place some 40,000 of them, one directly behind another, to get the same depth of colour; yet it would be essential to be able to apply the test to a single mitochondrion. It is clear that the test must give an intense colour-reaction even with very thin layers of the substance for which it is positive.

After a considerable amount of experiment, it was found best to apply the substances under test to the surfaces of cigarette paper. The paper used throughout this investigation is called 'Papier A.G.'; it is particularly thin and smooth. The substance under test was dissolved at high concentration in a suitable volatile solvent. The most useful solvents were chloroform (alone or mixed with methyl or ethyl alcohol or with pyridine), ether, ethyl alcohol (absolute or dilute), and water. Some substances (e.g. blood serum) required no solvent. It was necessary to avoid the presence of undissolved particles, as these tended to cling to the paper and thus make too thick a layer. A strip of the paper was soaked in the solution for fifteen hours or longer. It was then removed, wiped across a glass edge to get rid of any obvious excess, and pinned up to dry. If a drop of fluid accumulated at the lowest point of the paper, it was

removed. When the solvent had evaporated, the substance was left as a deposit on the paper. It was now ready for subjection to the test.

In order to find what form the deposit took, some of the treated papers were fixed, stained, embedded in celloidin, and cut into sections at right angles to the surface of the paper. These sections showed that the substance is deposited in the form of layers on the two surfaces of the paper. In the case of egg-lecithin, the mean thickness of both layers together was found to be approximately  $10\mu$ . This is large compared with the thickness, e.g., of a mitochondrion, but it is a good deal thinner than can be obtained with frozen sections of pith containing lipine.

Various modifications of the Smith-Dietrich test were tried, until a technique was discovered that separates lipines from other kinds of substances occurring in plants and animals, except certain proteins. The pyridine extraction test (p. 457) separates lipines from these proteins.

The final technique obviously had to be one that was applicable not only to substances deposited in thin layers on paper but also to tissues. Some of the necessary reagents penetrate slowly into tissues, and the periods of treatment must therefore be prolonged.

The lipines used in this investigation were lecithin, cephalin, sphingomyelin, and galactolipine, all obtained from the brains of sheep by Weil's method (1930), and also commercial egg-lecithin. Since the cephalin was obtained from brains, it must presumably have consisted largely of the serine ester (see Folch and Schneider, 1941).

#### Differences between the New Test and the Smith-Dietrich.

The new test differs from the Smith-Dietrich in the following particulars (as well as in others of minor importance):

- (1) Formaldehyde-calcium (see Baker, 1944, and p. 446 below) is substituted for the simple formaldehyde solution as fixative. The calcium ions immobilize the lipines.

(2) Potassium dichromate probably plays two roles in the Smith-Dietrich test: first, it preserves the lipines by oxidation; secondly, it leaves a basic compound in them which acts as a mordant and subsequently combines with haematein. In the new test these roles are to some extent separated. The preservative effect is achieved by transferring the tissue directly from formaldehyde-calcium to a solution containing potassium dichromate (instead of sectioning first). I attribute the success of the method with lecithin to the preservation of this substance by immediate treatment with dichromate after formaldehyde-calcium. When sections have been cut, they are treated with potassium dichromate, and any loss of this substance in the processes of washing before microtomy is thus made good. The second treatment with dichromate makes sure that the lipines are thoroughly mordanted. Kaufmann and Lehmann showed that the dichromate should be used not warm, but hot (60° C.). In the new technique the tissue is transferred from formaldehyde-calcium to a cool dichromate solution, so that it may be thoroughly penetrated by this before a high temperature is applied. It is then treated with a hot dichromate solution and the sections are mordanted in the same solution.

(3) The dye (acid haematein) is very carefully standardized in concentration. In the Smith-Dietrich test the haematoxylin used is allowed to 'ripen' naturally over a period of six months. A standard concentration of the oxidized product cannot be obtained in this way. It might be thought best simply to dissolve haematein at a known concentration, but unfortunately different specimens of haematein differ greatly in their capacity to stain lipines (probably because some of them are partly over-oxidized to a substance that is not a dye). In the new test haematein is prepared at standard concentration on the day of use, by oxidation of haematoxylin with sodium iodate.

(4) The differentiator is used at low concentration (to ensure even action), at a controlled temperature, and for a definitely specified period.

(5) The positive reaction is blue, blue-black, or grey (seldom quite black).



(6) A second test (the pyridine extraction test) is performed in order to separate lipines from certain proteins that react positively to the acid haematein test.

### Detailed Description of the Test.

The new test, as described in detail below, is applicable both to tissues and to known substances deposited on paper in the way described on p. 443. The only difference is that tissues have to be sectioned, while the known substances applied to paper are so thin that sectioning is unnecessary. It cannot be too strongly stressed that it is essential to adhere to the stated concentrations, temperatures, and periods; otherwise no certain conclusions can be drawn from the test. (See, however, p. 466.) The suggested times of day will be found convenient.

The following fluids are required:

#### Formaldehyde-calcium.

Formalin (formaldehyde of about 40 per cent.) . . . . .	10 c.c.
Calcium chloride (anhydrous), 10 per cent. aqueous . . . . .	10 c.c.
Distilled water . . . . .	80 c.c.

Keep powdered chalk in the solution. The solution is stable.

#### Dichromate-calcium.

Potassium dichromate . . . . .	5 gm.
Calcium chloride (anhydrous) . . . . .	1 gm.
Distilled water . . . . .	100 c.c.

The solution is stable. (A small precipitate may be neglected.)

#### Gelatine for Embedding.

Cresol, 0.25 per cent. aqueous solution . . . . .	100 c.c.
Finest gelatine . . . . .	25 gm.

Soak the gelatine for an hour in the cresol solution; then warm until the gelatine dissolves and strain through muslin while still warm. The gel is stable.

#### Acid Haematein.

Place 0.05 gm. of haematoxylin in a flask. Add 48 c.c. of distilled water and (exactly) 1 c.c. of a 1 per cent. aqueous solution of sodium iodate. Heat until the water just begins to boil. Cool. Add 1 c.c. of glacial acetic acid. Use on the day of preparation. (The special haematoxylin sold by the British Drug Houses for the measurement of pH was used in this investigation.)

## Borax-ferricyanide.

Potassium ferricyanide . . . . .	0.25 gm.
Borax (sodium tetraborate crystallized with ten molecules of water and powdered) . . . . .	0.25 gm.
Distilled water . . . . .	100 c.c.

The solution is stable if kept in a refrigerator.

- 1st day
- 10 a.m. I. Fix in formaldehyde-calcium for 6 hours.
- 4 p.m. II. Without washing, transfer to dichromate-calcium at room temperature and leave for 18 hours.
- 2nd day
- 10 a.m. III. Transfer to dichromate-calcium in a paraffin oven at 60° C. and leave for 24 hours.
- 3rd day
- 10 a.m. IV. Either wash for 6 hours in running water and pass on to stage V, or wash overnight in running water (omitting stages V, VI, and VII), and pass on directly to stage VIII.
- 4 p.m. V. Transfer to melted gelatine in an incubator at 37° C. and leave overnight.
- 4th day
- About 10 a.m. VI. Solidify the gelatine in a refrigerator. Cut out a rectangular block containing the tissue and leave it overnight in formaldehyde-calcium to make the gelatine insoluble.
- 5th day
- About 9.30 a.m. VII. Wash the block in running water for half an hour.
- About 10 a.m. VIII. Cut sections on the freezing microtome at 10 $\mu$ .
- 10.55 a.m. IX. Put the loose sections into dichromate-calcium at 60° C. in the paraffin oven and leave for 1 hour.
- 11.55 a.m. X. Wash the sections in several changes of water for 5 minutes. The last wash should be in distilled water.
- 12 midday XI. Put the sections into acid haematein at 37° C. in the incubator. Leave for 5 hours.
- 5 p.m. XII. Rinse the sections with distilled water and leave them for 18 hours in borax-ferricyanide at 37° C. in the incubator.

6th day

11 a.m. XIII. Wash the sections for 10 minutes in several changes of water. The last wash should be in distilled water.

11.10 a.m. XIV. Mount in Farrant's medium or in glycerine-jelly (or in balsam, by the method given on pp. 449-450).

Result: lipines and certain proteins, blue, blue-black, or grey. (Feeble reactions, such as a very pale dirty blue, are regarded as negative.) The background is pale yellow.

Notes on Technique.—The actual object to be investigated should be exposed to the action of the reagents as fully as possible before fixation begins. (This is often difficult with plant tissues, as the cell-walls interfere with the penetration of the fluids.) The piece of tissue should not exceed 3 mm. in thickness. It should lie on glass wool during stage I, so that the fixative may act from all sides. During this stage the capsule must contain an excess of precipitated chalk, to prevent the development of acidity during fixation. (This applies also when gelatine blocks are being hardened or stored in formaldehyde-calcium; but when sections are stored in this fluid, a small chunk of solid chalk should be used instead, as the precipitated powder tends to adhere to sections.)

No fluid used in the test should be used again on a subsequent occasion. All fluids used at stated temperatures must be already at these temperatures when the tissue or section is placed in them. (A fluid placed in a capsule on the floor of a paraffin oven often shows a temperature a few degrees higher than that of the air in the oven. For the purpose of this test the small difference does not matter.)

It is often said to be difficult to cut frozen sections at less than  $15\mu$ , but there is actually no difficulty in cutting  $10\mu$  sections of gelatine-embedded material. When a number of sections have been cut, it sometimes happens that a few of them look 'better' than the rest; these are thick sections, arising from a change in the temperature of the block between the cutting of one section and the next, and they must be discarded. When it has been shown by means of  $10\mu$  sections

that there is no massive body in the tissue giving a positive reaction to the test, it is legitimate to cut sections of the same material at  $15\mu$ .

Difficulty may be experienced in handling the sections, as they are sometimes sticky at stages IX and X. If so, it is better to lift them with the tip of a fine mounted needle than with a seeker or bent glass rod; but the best plan of all is to put the freshly cut sections into a porcelain vessel with a finely perforated bottom, standing in a capsule of water. Move the porcelain vessel from one fluid to another in stages IX to XIII. The sections may stick to the porcelain in stages IX and X, but they will be loose (or easily loosened) when stage XIII is reached.

It is essential that the sections should be bathed in plenty of fluid during stages XI and XII, because both stain and differentiator are very dilute. The volume should be such that if it is increased, no appreciable effect will be noticed. This is achieved by allowing about 10 c.c. to each section. When a perforated porcelain vessel is used, there must be at least this amount of fluid within the porcelain, as the fluid outside the perforations has little effect.

If it is inconvenient to attend to the test over six consecutive days, either the gelatine block (stage VI) or the sections (stage VIII) may be stored in formaldehyde-calcium for a few days. This has no noticeable effect on the test. If storage is prolonged for weeks or months, the subsequent staining reaction appears to be intensified. I have not noticed that objects that are ordinarily negative become positive under these circumstances, but it is advisable to avoid long storage in critical work. After storage, wash in running water or repeated changes.

Farrants's is generally the best mounting medium. Put the section on the slide, cover it with a mixture of equal volumes of glycerine and water, leave it for a few minutes, remove the excess of fluid with a cloth, and mount.

When once the lipines have been stained, no lipid-solvent whatever (not even pyridine at  $60^{\circ}\text{C}$ ., or a boiling mixture of methyl alcohol and chloroform) will remove the stain. It is therefore legitimate to mount in balsam, but the necessary

dehydration distorts the gelatine considerably unless precautions are taken. At the end of stage XIII the section may be attached to a glass slide with gelatine, by the method given in my 1944 paper. It can then be dehydrated and mounted as though it were a paraffin section. Sections should not be attached to slides before staining, as they almost inevitably come loose in the borax-ferricyanide.

### The Results of the Application of the Test to known Substances.

The composition of the reagents used in the test, the temperatures at which they are used, and the times during which they act, were determined by experiments carried out on known substances deposited on paper in the way described above (p. 443). The object was to devise a test that would give a positive colour-reaction with lipines but not with any other organic substances occurring in plants and animals. This object was achieved, except that certain proteins also give a positive reaction. As was mentioned above, lipines are separated from these proteins by the application of the pyridine-extraction test, which is described on pp. 457-8.

The substances listed below were chosen as being representative of most kinds of substances occurring in plants and animals. In a few cases the actual substances present in organisms were represented by closely allied substances that are easier to procure: for instance, the particular paraffins of plant cuticles were represented by the ordinary paraffins of the laboratory. A few substances (e.g. phenol) were also tested because their compounds occur in organisms.

The colours given in the list were recorded by my friends, Mr. D. A. Kempson and Mr. P. A. Trotman. Having passed the impregnated pieces of paper through the test and mounted them in glycerine-jelly on microscopical glass slides, I asked Mr. Kempson and Mr. Trotman to judge the colour of each. The slides were marked only with numbers, and related substances were often given widely different numbers. The significance of the numbers was not communicated to the judges, who were entirely ignorant of the purpose of what they were

doing until they had made their judgments. These precautions were taken so as to prevent any possibility of the action of conscious or unconscious bias.

In a number of cases the test was performed repeatedly with a single substance. When the results were different, they were all recorded and are noted below. The differences were due to the substance having been dissolved at different concentrations, so that there was more or less of the substance on the paper in different cases.

	LIPIDS
Paraffins.	
Medicinal paraffin.	Almost unstained.
Paraffin, m.p. 48° C.	" "
Fatty Acids.	
n-butyric acid.	" "
Palmitic acid.	" "
Stearic acid.	" "
Oleic acid.	" "
Ricinoleic acid.	Very pale dirty blue, almost unstained.
Triglycerides.	
Tributylin.	Almost unstained.
Tristearin.	" "
Triolein.	" "
Fatty Waxes.	
Carnauba wax.	Very pale blue, almost unstained.
Beeswax (white).	Very pale dirty blue, opaque whitish.
Spermaceti.	Almost unstained.
Lipines.	
Phospholipines.	
Egg lecithin.	Dark blue.
Brain lecithin.	Dark blue, dark blue, medium blue.
Cephalin.	Blue-black, blue-black, dark blue, pale blue, greenish blue.
Sphingomyelin.	Dark blue.
Galactolipines.	
Brain galactolipine.	Dark blue, medium blue, medium blue, pale blue, pale blue, pale blue, very pale dirty blue.

## Sterols and their Esters.

Cholesterol.	Opaque whitish.
Cholesteryl oleate.	Almost unstained.
Lanoline.	" "
Ergosterol.	" "

## Carotenes.

Carotene.	Pale brown.
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## NON-LIPOIDS

## Aliphatic Alcohols and their Derivatives.

Glycerol.	Almost unstained.
Sodium glycerophosphate.	" "
Sorbitol.	" "

## Phenols and their Derivatives.

Phenol.	Almost unstained.
Cresol.	" "
Adrenalin hydrochloride.	" "

## Organic Acids other than Fatty Acids.

Benzoic acid.	Almost unstained.
Citric acid.	" "
Oxalic acid.	" "
Tannic acid.	" "

## Soaps.

Sodium palmitate.	" "
Sodium stearate.	" "
Sodium oleate.	Ivory.
Potassium oleate.	Almost unstained.
Sodium ricinoleate.	Very pale dirty blue.

## Essential Oils and Oleo-resins, and their Constituents.

Pinene.	Almost unstained.
Eugenol.	" "
Menthol.	" "
Vanillin.	" "
Turpentine.	" "
Organum oil.	" "
Clove oil.	" "
Cedarwood oil.	" "
Canada balsam.	Opaque whitish.

## Carbohydrates and Related Substances.

Dextrose.	Almost unstained.
Lactose.	" "
Sucrose.	" "
Dextrine.	" "
Glycogen.	" "
Starch (soluble).	" "
Cellulose.	" "
Gum arabic.	" "
Gum mastic.	" "
Tragacanth.	" "
Agar.	" "
Chitin.	See p. 454.
Lignin.	See p. 454.

## Glucosides.

Digitonine.	Almost unstained.
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## Amino-acids, Proteins, Enzymes, Products of Protein Metabolism.

Cysteine hydrochloride.	Almost unstained.
Fibrinogen.	Very pale dirty blue, almost unstained.
Caseinogen.	Dark blue, very pale dirty blue, very pale dirty blue.
Legumin.	See p. 454.
Collagen.	See p. 454.
Gelatine.	Black, dark brown, dark brown.
Egg-white.	Pale brown (border of paper darker), ditto, very pale dirty blue, ditto.
Blood-serum (rabbit).	Bluish brown, very pale dirty blue.
Blood-plasma (fowl).	Light bluish brown, ivory.
Blood albumen.	Bluish brown, light bluish brown.
Mucin.	Dark blue, pale brown (border of paper blue), very pale dirty blue, almost unstained.
Nucleoprotein (from thymus gland).	Bluish brown, pale brown (border of paper blue).
Haemoglobin.	Medium grey.
Pepsin.	Almost unstained.
Trypsin.	Very pale dirty blue, almost unstained.
Urea.	Almost unstained.

## Purine Derivatives and their Compounds.

Guanine.	Almost unstained.
Uric acid.	" "
Caffeine.	" "



**Purine Derivatives and their Compounds (cont.).**

Nucleic acid.	Very pale dirty blue.
Nucleic acid (sodium salt).	Almost unstained.
Ribonucleic acid.	" "

**Unclassified.**

Chlorophyll.	Green.
Pyridine.	Almost unstained.
Nicotine.	" "

Four substances—chitin, lignin, legumin, and collagen—were tested as they occur in actual tissues, instead of being dissolved and deposited on cigarette paper. The exoskeleton of *Gammarus pulex* was used for the test of chitin. The whole animal was sectioned transversely, and the chitin was thus presented in the full thickness of the  $10\mu$  sections. It was completely negative to the test. Lignin was tested in  $10\mu$  transverse sections of the leaf of the shepherd's purse (*Capsella Bursa pastoris*) and cherry-laurel (*Prunus Lauro-cerasus*). The xylem was either colourless or nearly so. If thick sections are used, a pale blue with a reddish tinge is seen in the xylem; but the test is only applicable to objects  $10\mu$  or less in thickness. Legumin was tested in sections of the cotyledon of the seed of the broad bean (*Vicia Faba*). The protein granules in the parenchyma cells became black or blue-black. Collagen was tested in  $10\mu$  sections of the tunica albuginea of the testis of the mouse. It was completely negative.

A glance at the list given above shows that the acid-haematein test separates the lipines sharply from representatives of all other groups of lipoids: a medium or dark blue or blue-black reaction is never given by any lipid other than a lipine. (The very pale blue given in a few cases may have been due to the presence of traces of lipine in the substances tested.) Among the lipines themselves, a positive reaction is always given by phospholipines, unless the substance was deposited from a dilute solution, in which case a medium blue or pale blue reaction is given. (The greenish blue given in one instance by cephalin was due to the interference of the yellowish brown colour of the specimen used.) Galactolipine, however, generally

gives a pale-blue reaction, and it is quite likely that a pure specimen of this substance would give no blue reaction at all. Unfortunately it is difficult to free galactolipine completely from sphingomyelin, which gives a strongly positive reaction; and such colour as develops may be due to sphingomyelin. Further tests are being made to settle this point. If pure galactolipine is found to be negative, then the acid-haematein test is a test for phospholipines, not for all lipines.

Sphingomyelin appears to give a stronger reaction than cephalin, and cephalin than lecithin. This has not been definitely established, however, by a quantitative study.

The nature of the blue compound formed with lipines is not known. If further investigation shows that pure galactolipine is negative, it will become probable that the phosphoric acid of the lipines provides a link with calcium and/or a basic substance derived from potassium dichromate, and that the compound thus formed gives a lake with haematein.

When any substance gives a negative reaction, this may be due to one of three causes. (1) It may be a substance that is not rendered insoluble by the fixative. It therefore dissolves out and never meets the haematein. (2) It may be a substance that does not react with the mordants to give a compound that forms a blue lake with haematein. (3) It may be capable of forming a blue lake, but be present in too small amount for the result to be visible. A negative result must never be taken to prove the actual absence of lipine.

Inorganic substances have not been tested. Any substance that gives a blue lake with haematein would necessarily give a positive reaction unless dissolved out before meeting the dye. If the test is found to give a positive reaction with the material of spicules or other skeletal substances, or with matter encrusting chitin, the possibility that inorganic matter may be the cause must be kept in mind. In all cases of doubt the test should be repeated with the omission of any treatment with calcium chloride or potassium dichromate at any stage. If a positive reaction is obtained under these circumstances, there is no evidence of the presence of lipine.

The test is negative for every kind of organic non-lipoid that

was tested, except certain proteins. Legumin, caseinogen, and mucin have a tendency to give a positive reaction, and other proteins sometimes give a brownish colour with an admixture of blue. The three proteins that have the greatest tendency to react positively are markedly acidic in character.

### General Remarks.

When an organic substance occurring in the tissues of a plant or animal gives a positive reaction to the acid-haematein test, there are three possibilities: the substance contains lipine, or protein, or both. Now the whole purpose of the test is to disclose the presence of lipine. It might be thought sufficient to test with a lipoid-soluble colouring agent such as sudan black: if the object gave a positive reaction both to sudan black and to the acid-haematein test, it could be regarded as containing lipine. This reasoning, however, is false, for two reasons. First, acid haematein is more intense than sudan black, and will show lipine where sudan black will not. One would be limiting oneself unnecessarily if one restricted the acid-haematein test to objects that are positive to sudan black. Secondly, a lipoid other than lipine might be intimately associated with a positively-reacting protein; in such a case a positive reaction would be given both by sudan black and by acid haematein, yet lipine would be absent.

An alternative method would be to test for protein, and only to accept a positive reaction with the acid-haematein test as proving the presence of lipine in cases in which the protein test gave a negative result. This, however, does not resolve the difficulty, for three reasons. First, the ordinary tests for proteins give very feeble colours (except with the storage protein granules of plants), and are not applicable to minute cell-inclusions. Secondly, an object in a cell is surrounded by proteins on every side, and no test for proteins could show that they were absent from a small cell-inclusion. One could only say that there was no evidence that it contained more protein than the surrounding protoplasm. Thirdly, one would be limiting oneself unnecessarily if one regarded the test as applicable only to lipines that are not intimately associated with protein.

For these reasons, the ordinary tests for lipoids and for proteins will not resolve the difficulty. All my attempts to invent a test that would be very sensitive to lipine but negative to all proteins have failed. The attempt was therefore made to find a way of treating tissues that would leave unaltered (or even increase) the tendency of the acid-haematein test to colour proteins, while destroying its capacity to colour lipine. This attempt was successful. The pyridine-extraction method, described below, permits a sharp separation of lipines from positively reacting proteins.

### THE PYRIDINE-EXTRACTION TEST

#### Introduction.

This is really a variant of the acid-haematein test. The full name should be the acid-haematein test following pyridine extraction, but it is convenient to call it the pyridine-extraction test. This test increases the tendency of proteins to give a positive reaction, while eliminating lipines.

The tissue is first fixed with a fluid resembling Bouin's, but weaker. The purpose of this fixative is to leave the tissue in such a state that lipines are particularly easily extracted afterwards by solvents. Many different fixatives would be suitable if the test were only applicable to papers on which pure substances had been deposited; but the lipines in the tissues are often present as lipoprotein complexes, and certain fixatives act upon the protein component in such a way as to prevent the release of the lipine. Bouin's fluid was found empirically to render the lipines rather easily extracted, except in the outermost cells of the piece of tissue. It was found that reduction in the concentration of the formaldehyde and of the picric acid improved the fluid in this respect, and 'weak Bouin's fluid' resulted.

Various solvents were tried, and pyridine at 60° C. was found to be the most effective.

In the pyridine-extraction test the tissue is first fixed in weak Bouin's fluid and then washed and extracted with hot pyridine. It is then washed again and carried through the standard acid-haematein test from stage II onwards.

## Detailed Description of the Test.

'Weak Bouin's fluid' is as follows:

Picric acid, saturated aqueous solution . . . . .	50 c.c.
Formalin (commercial, not diluted nor neutralized) . . . . .	10 c.c.
Glacial acetic acid . . . . .	5 c.c.
Distilled water. . . . .	35 c.c.

The fluid is stable.

## 1st day

2 p.m. I. Fix for 20 hours in weak Bouin's fluid.

## 2nd day

10 a.m. II. Transfer to 70 per cent. alcohol for 1 hour.

11 a.m. III. Transfer to 50 per cent. alcohol for  $\frac{1}{2}$  hour.

11.30 a.m. IV. Wash for  $\frac{1}{2}$  hour in running water (or several changes).

12 midday. V. Transfer to pyridine at room temperature and leave for 1 hour.

1 p.m. VI. Change the pyridine and leave for 1 hour.

2 p.m. VII. Transfer to pyridine at 60° C. (in the paraffin oven) and leave for 24 hours.

## 3rd day.

2 p.m. VIII. Wash for 2 hours in running water (or repeated changes).

4 p.m. IX. Transfer to dichromate-calcium at room temperature. Now proceed with the usual acid-haematein test, beginning at stage II.

Notes on Technique.—Particular care should be taken to expose the object under investigation thoroughly, so that the fluids may have ready access to it. The piece of tissue should not exceed 3 mm. in thickness. During stages I and VII it should lie on a wisp of glass wool, so that the fluids may reach it from all directions.

## The Results of the Application of the Test to known Substances.

The pyridine-extraction test was applied to the relevant substances (i.e. those that give a positive reaction to the acid-haematein test). The substances were deposited on cigarette

papers in the way already described. The colours were judged, as before, by Mr. Kempson and Mr. Trotman. The results were as follows:

#### Lipines.

##### Phospholipines.

Egg lecithin.	Unstained.
Brain lecithin.	"
Cephalin.	"
Sphingomyelin.	"

##### Galactolipines.

Brain galactolipine.	"
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#### Proteins.

Caseinogen.	Medium blue.
Legumin.	See below.
Mucin.	Blue-black.
Nucleoprotein (from thymus gland).	Dark blue.

Legumin was tested in sections of the cotyledon of the seed of the broad bean. The protein granules in the parenchyma cells showed dark blue rims.

Gelatine behaves anomalously. It usually gives a brown reaction to both the acid-haematein and pyridine-extraction tests; but when present in thick layers, it shows a black reaction to the former. However, it need not be considered further, since it does not occur in tissues. Collagen, as we have seen, is completely negative to the acid-haematein test.

The results given above show that the pyridine-extraction test sharply separates lipines from those proteins that have a tendency to give a positive reaction to the acid-haematein test. Every substance falls into one of four groups:

A. Substances that give a positive (blue, blue-black, or grey) reaction to both the acid-haematein and pyridine-extraction tests. (Caseinogen is an example.)

B. Substances that are negative to the acid-haematein test but positive to the pyridine-extraction test. (Chromatin in thin layers is an example. In relatively thick layers it is faintly positive to the acid-haematein test.)

C. Substances that are positive to the acid-haematein test but negative to the pyridine-extraction test. These substances are lipines. (Possible sources of invalidity in the tests are mentioned below.)

D. Substances that are negative to both the acid-haematein and pyridine-extraction tests. (Ground cytoplasm is an example.)

It is not claimed that substances in group A are necessarily proteins. A lipine that was so firmly bound to a negatively reacting protein that it resisted pyridine extraction would fall into group A. If one relied on insolubility in pyridine as an index that the substance concerned was a protein, one would be falling into the error of supposing that insolubility can be used as a criterion in the histochemical recognition of substances occurring in tissues. A substance that is readily soluble in the pure state in a given solvent may fail to be extracted by that solvent under the circumstances in which it occurs in the tissues of an organism, particularly after fixation. For this reason no histochemical conclusion is drawn about substances falling into group A. It is only when the solvent does work, not when it does not, that a conclusion is reached. When pyridine does extract an organic substance that is capable of reacting positively to the acid-haematein test, then that substance is a lipine; for the organic non-lipines that react positively to that test have no tendency to be extracted by pyridine under the conditions of the experiment. On the contrary, they remain and are coloured blue.

#### POSSIBLE SOURCES OF INVALIDITY IN THE TESTS

The validity of the tests depends on the results of their application to known substances deposited on cigarette paper. It is believed that their validity, established in that way, is as great as that of most other histochemical tests that are regarded as valid. The purpose of this section is to point out a few conceivable sources of invalidity. It is left to other histochemists to assess their weight.

A substance that is not a lipine nor a protein may exist which would nevertheless react positively to the acid-haematein test under certain circumstances, but does not do so in the test

with substances deposited on papers for one of the following reasons:

(1) The substance might be so little soluble in the solvent used that not enough of it was deposited on the paper.

(2) A volatile substance might evaporate before the paper was placed in the formaldehyde-calcium. (This could only apply to some of the essential oils and their constituents.)

(3) A substance may exist that is dissolved during fixation when the acid-haematein test is applied to substances deposited on paper, but which is retained in tissues by adsorption on some other substance.

(4) An inorganic substance that was not dissolved out in the course of the test might give a blue lake with haematein. (See p. 455.)

(5) Substances may exist which, though individually negative to the test, give a positive reaction when mixed together.

The pyridine-extraction test might be invalidated by one of these circumstances:

(1) A protein may exist that reacts positively to the acid-haematein test but is dissolved out by pyridine extraction.

(2) A protein may exist that reacts positively to the acid-haematein test but fails to do so after pyridine extraction, though still present in the tissue.

It must be repeated that the test would not be invalidated if a lipine were made resistant to pyridine extraction through association with a negatively reacting protein. In such circumstances a positive reaction would be given to both tests, and therefore no conclusion as to chemical composition would be drawn. This circumstance would reduce the applicability of the test but not affect its validity.

## THE RESULTS OF THE APPLICATION OF THE TESTS TO TISSUES

### General Remarks.

It follows from what has gone before that in investigating the distribution of lipines in any tissue one first of all applies the acid-haematein test. If any object is seen to be coloured



blue, blue-black, or grey, the pyridine-extraction test is next applied. If the object that reacted positively to the acid-haematein test is now seen not to be coloured blue, blue-black, or grey, then it consists of or contains lipine. If, however, it is blue, blue-black, or grey after the application of the pyridine-extraction test, then no conclusion can be drawn about its chemical composition.

When any object is very small (e.g. a mitochondrion), it is essential to use an immersion objective and to focus very carefully before making any judgment about the colour. White light should be used (e.g. by the use of a 'daylight' screen). When the cytoplasm contains many positively reacting minute objects, the appearance under the 4 mm. objective is so pale that at a casual glance one would regard the reaction as too feeble to serve as the basis for a sound judgment. If, however, a single one of the minute objects is carefully focused under the immersion objective, it may be seen to give an undeniably positive reaction. A little practice is necessary, for one may not have much experience in judging the colour of objects that subtend such a small angle at the eye as a single mitochondrion does, even when an immersion objective and high-power eyepiece are used.

The difference between the results of the acid-haematein and pyridine-extraction tests is most striking. The picture given by the acid-haematein test is unfamiliar, and it is not always easy at first to identify the various objects in the section. Nuclei are very pale yellow and contain no sign of chromatin; for although experiments with cigarette papers impregnated with nucleo-protein sometimes give a feeble trace of a positive reaction, the amount of nucleo-protein present in nuclei is too small to give any sign of blueness. Mitochondria, on the contrary, are often definitely blue, blue-black, or grey, and sometimes quite intensely coloured. Various secretion droplets, &c., which are not made obvious in a routine histological preparation, may also stand out boldly. The whole appearance is changed when the pyridine-extraction test is applied. The mitochondria, &c., have now been eliminated; but acidic proteins, and particularly nucleo-proteins, give a strongly positive

reaction. The result is that the section somewhat resembles a routine preparation that has been stained with Ehrlich's haematoxylin and very feebly counterstained with a yellow dye. Plate 10 shows two examples of the contrast. The photomicrographs on the left represent sections of tissues that have been subjected to the acid-haematein test, while those on the right represent the same tissues after passage through the pyridine-extraction test. In the latter little can be seen except chromatin and red blood corpuscles.

The general fixation given by both tests is quite good. The only exception is provided by myelin, which, in the acid-haematein test, gives the artificial appearance known as 'the mitochondria of the myelin' (Nageotte, 1922). The resemblance to the figures on p. 230 of Nageotte's book was pointed out to me by Dr. F. K. Sanders.

A few of the results obtained with the acid-haematein and pyridine-extraction tests are tabulated below as illustrations. A general survey of the distribution of lipines in plant and animal tissues is made possible by the invention of these tests, but is not undertaken in the present paper. In the Table the various tissue-constituents are placed in four groups labelled A, B, C, and D, in accordance with the classification given on pp. 459-460. Those in group C are the ones that consist of or contain lipine. No certain conclusions can be drawn about the objects in groups A, B, and D.

The mitochondria of certain cells, as we have already seen, contain lipine. They are further considered on p. 466. The middle-piece of the spermatozoon of the mouse, which is largely of mitochondrial origin, is also seen to contain lipine. The secretion droplets in the interstitial cells of the testis react so strongly that a striking appearance is given even under the low power of the microscope. It is interesting to find concrete evidence that the secretion droplets of the Paneth cells in the intestine contain lipine. It was only to be expected that myelin would be shown to contain it.

The plasmosomes (or nucleoli, in the narrower sense of that word) are particularly interesting. If a section is observed from time to time during the period while the potassium ferricyanide

*Table showing the results of the application of the acid-haematein and pyridine-extraction tests to certain objects*

	<i>Acid-haematein test</i>	<i>Pyridine-extraction test</i>
A. Cytoplasm of red blood corpuscles of frog . . . . .	Positive	Positive
Red blood corpuscles of mouse . . . . .	Positive	Positive
Chromatophors in leaf of cherry-laurel . . . . .	Positive	Positive (weakly)
Protein granules in parenchyma cells of cotyledon of bean . . . . .	Positive	The rims strongly positive
B. Chromatin in various cells of frog . . . . .	Negative	Positive
Chromatin in various cells of mouse . . . . .	Negative	Positive <sup>1</sup>
C. Mitochondria in cells of convoluted tubules in kidney of frog . . . . .	Positive (intensely)	Negative <sup>2</sup>
Mitochondria in cells of convoluted tubules in kidney of mouse . . . . .	Positive (intensely)	Negative <sup>2</sup>
Mitochondria in liver cells of mouse . . . . .	Positive	Negative <sup>2</sup>
Mitochondria in intestinal epithelial cells of mouse . . . . .	Positive	Negative <sup>2</sup>
Mitochondria in primary spermatocytes of mouse . . . . .	Positive (rather weakly)	Negative
Mitochondria in late spermatids of mouse ('von Ebner's granules') . . . . .	Positive	Negative
Middle pieces of spermatozoa of mouse . . . . .	Positive	Negative <sup>2</sup>
Secretion droplets in interstitial cells of testis of mouse . . . . .	Positive (very intensely)	Negative
Secretion droplets in Paneth cells in intestine of mouse . . . . .	Positive (rather weakly)	Negative
Myelin in sciatic nerve of rat . . . . .	Positive	Negative <sup>4</sup>
Plasmosomes (nucleoli) in cells of convoluted tubules in kidney of frog . . . . .	Positive	Negative (except perhaps the rims)
D. Ground cytoplasm of various cells of frog and mouse . . . . .	Negative	Negative
Collagen in tunica albuginea of testis of mouse . . . . .	Negative	Negative
Mucin in goblet-cells of intestine of mouse . . . . .	Negative	Negative

<sup>1</sup> Strongly positive in cells of testis and kidney, weakly in liver cells.

<sup>2</sup> Traces of a positive reaction in the most external cells of the piece of tissue.

<sup>3</sup> Traces of a positive reaction in a few tubules.

<sup>4</sup> Positive in the centre of the nerve.

is acting, it will be found that the plasmosomes often retain a blue colour for a long time, even though they usually lose it before the eighteen hours are complete. This suggests, without proving, that they contain a certain amount of lipine (see p. 466). In certain cells of the frog, however, and particularly in those of the convoluted tubules of the kidney, the positive reaction is given after the full period of differentiation. They are negative to the pyridine-extraction test, except that here and there their rims are coloured blue; but this is apparently due to the precipitation of nucleo-protein on them. The plasmosomes are thus shown to contain lipine. Fels (1926) claimed to have demonstrated that the plasmosome of the oocyte of the rabbit sometimes contains lipine. This conclusion, however, was not reliable, for he used a modification of the Smith-Dietrich test without proving the validity of the modification by experiments on known substances. Further, he decided when to stop differentiating by the use of an arbitrarily chosen criterion (the decolorization of chromatin).

Since the plasmosomes are known from micro-incineration studies to contain iron, it might be thought that treatment with formaldehyde-calcium and dichromate-calcium releases iron from combination, and that this metal subsequently gives a lake with haematein. To test this possibility, frog tissues were carried through the acid-haematein test as usual up to the stage of sectioning; some of the sections were then soaked in concentrated ammonium sulphide solution, washed, and treated with acid potassium ferricyanide, while other sections were treated with acid potassium ferrocyanide solution. These tests, for ferrous and ferric iron respectively, gave negative results, and it is therefore clear that the blue reaction with the acid-haematein test is not due to the release of iron from combination. (By the same tests it was proved that the cytoplasm of the red blood corpuscles of the frog and mouse do not release iron from haemoglobin on subjection to the acid-haematein test. The positive reaction may be due to protein, for it appears also in the pyridine-extraction test.)

Mucin has some tendency to give a positive reaction when present in relatively thick layers on cigarette paper, but, as

the Table on p. 464 shows, it is negative when it occurs in the form of small globules in the goblet-cells of the intestine.

When an object in a tissue gives a negative reaction to the acid-haematein test, it is legitimate to repeat the experiment on another section, reducing the period in the borax-ferricyanide by several hours. If a positive reaction is now given, while the same object reacts negatively to the pyridine-extraction test, it may be regarded as likely that the object contains lipine. Other tests should then be applied for the purpose of confirmation, such as those described in an earlier paper (Baker, 1944). It must be stressed, however, that the acid-haematein and pyridine-extraction tests only provide a reliable indication of the presence of lipine if they are carried out in exact conformity with the detailed instructions.

When the acid-haematein test is used simply as a method for showing mitochondria, and not as a histochemical test, it is usually best to reduce the period in borax-ferricyanide by several hours.

### The Chemical Composition of Mitochondria.

Ever since Regaud (1909) first pointed out that the staining reactions of mitochondria are similar to those of myelin, there has been strong reason for supposing that they contain lipine. Strict histochemical proof has, however, been lacking. The Smith-Dietrich test gives a negative result, as anyone can prove for himself by applying Dietrich's directions to mammalian liver or kidney-cortex, where the mitochondria are relatively large and particularly easy to study. Since no other test for lipines, hitherto available, is as dependable as the Smith-Dietrich, we have been left with no positive proof from *in situ* studies that mitochondria contain lipine. By far the best evidence on this subject hitherto available comes from the direct analysis of material that can be obtained by centrifuging mashed tissues and is thought to consist of mitochondria. In the pioneer work of Bensley and Hoerr (1934), too low a figure was obtained for the lipine-content of this material. More recent studies by various authors suggest that about 25 per cent.

of it is lipid, and that some 55 per cent. of this is phospholipine (see especially Claude, 1941, and Hoerr, 1943). This would be conclusive if there were positive proof that the material analysed actually consists of mitochondria. The evidence is strong, but would not appear to be absolutely conclusive.

A few words must be devoted to the identification of mitochondria in mammalian liver. The objects that are ordinarily supposed to be the mitochondria of liver-cells are larger than many mitochondria (though smaller than those of the convoluted tubules of the kidney), and Claude regards them as being of a different nature. He refers to them as 'large granules' and 'secretory granules', and suggests that they may be transformed mitochondria. In the present paper they are regarded as mitochondria, which they resemble in their form and staining reactions. Gland-cells that contain secretory granules also contain mitochondria, and if the objects usually identified as mitochondria in liver-cells are actually secretory granules, it is certainly surprising that the cells contain no mitochondria; for Claude's 'small granules' or 'microsomes' can scarcely be mitochondria, as they are generally too small to be seen under the microscope. (While differing from Claude in the identification of mitochondria in liver-cells, I should like to take this opportunity of paying tribute to his important work on the chemical analysis of the cell by centrifuge-studies.)

It is worth remarking that what are here called the mitochondria of liver-cells are unlike most secretory granules in possessing the form of longer or shorter rods. They are not spherical, though they readily become so when unsuitable fixatives are used. Mitochondria tend to swell up into large spheres unless a suitable indifferent salt is present in the fixative to counteract osmotic distortion. Potassium dichromate also swells rod-shaped mitochondria into spheres unless mixed with other substances that oppose this tendency. It may even be queried whether mitochondria are ever perfectly spherical during life, though they often appear so in fixed preparations. Studies of living cells show that the spherical form is at any rate rarer than one would judge from the examination of fixed preparations.

The Table on p. 464 shows that the mitochondria of diverse cells contain lipine. The generally held opinion is thus proved to be true by a rigorous histochemical test. The results provide support for the view that the supposed mitochondrial material derived from tissues by centrifuging does in fact consist of mitochondria.

No claim is made that the mitochondria of all cells respond positively to the acid-haematein test. Very small mitochondria do not do so. This is presumably because their minuteness puts them beyond the range of sensitiveness of the test.

#### ACKNOWLEDGEMENTS

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#### SUMMARY

1. The purpose of the investigation described in this paper was to find a rigorous and sensitive histochemical colour-test for the demonstration of lipine in plant and animal tissues.

2. Full details are given of two new tests, called the acid-haematein and the pyridine-extraction tests. The presence of lipine is reliably indicated by the use of the two tests in conjunction.

3. The acid-haematein test is derived from that of Smith and Dietrich, but unlike the latter gives a positive reaction with pure lecithin. It is also much more sensitive.

4. Diverse organic compounds that occur in the tissues of plants and animals were deposited on paper and subjected to the acid-haematein test. The test was shown to be negative to members of all the groups of such substances that were studied, except lipines and certain proteins.

5. The pyridine-extraction test serves to separate the positive reaction with lipines from that with certain proteins. Its validity was proved by experiments on known substances deposited on paper.

6. In the pyridine-extraction test, tissues are extracted with hot pyridine before subjection to the acid-haematein test. The tendency for certain proteins to give a positive reaction is now somewhat increased, while lipines are wholly negative.

7. Any organic substance that reacts positively to the acid-haematein test but negatively after pyridine extraction consists of or contains lipine.

8. Mitochondria are among the tissue-constituents that are shown by the tests to contain lipine. It has long been regarded as probable that they do so; but they respond negatively to the Smith-Dietrich test, and there has not previously been rigorous proof by any *in situ* histochemical method that they do in fact contain lipine.

## DESCRIPTION OF PLATE 10

A scale representing  $50\mu$  is placed at the top of the plate.

Fig. 1.—The liver of the mouse (acid-haematein test).

Fig. 2.—Ditto (pyridine-extraction test).

Fig. 3.—The kidney-cortex of the mouse (acid-haematein test).

Fig. 4.—Ditto (pyridine-extraction test).

In Figs. 1 and 3 (acid-haematein test) only the mitochondria and red blood corpuscles are stained, the nuclei appearing as unstained circles. In Figs. 2 and 4 (pyridine-extraction test) the mitochondria have been dissolved away, and only the chromatin and red blood corpuscles are stained.

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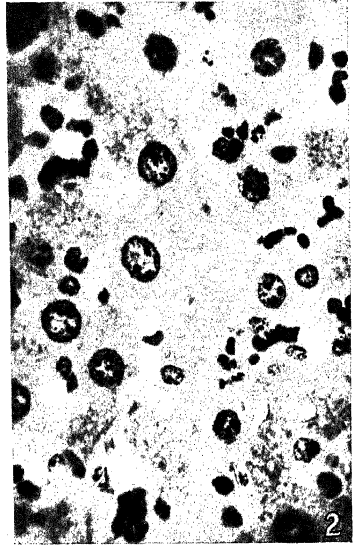
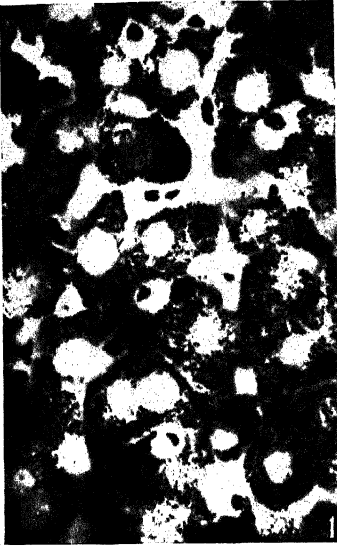
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